ASSESSMENT OF PERIOSTIN LEVELS IN GINGIVAL CREVICULAR FLUID OF PATIENTS WITH CHRONIC PERIODONTITIS AND AGGRESSIVE PERIODONTITIS AND COMPARISON WITH THE HEALTHY SUBJECTS

Dissertation submitted to

THE TAMILNADU Dr. M.G.R. MEDICAL UNIVERSITY

In partial fulfilment for the Degree of

MASTER OF DENTAL SURGERY

BRANCH II
DEPARTMENT OF PERIODONTICS

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CERTIFICATE

This is to certify that this dissertation titled **"ASSESSMENT OF PERIOSTIN LEVELS IN GINGIVAL CREVICULAR FLUID OF PATIENTS WITH CHRONIC PERIODONTITIS AND AGGRESSIVE PERIODONTITIS AND COMPARISON WITH THE HEALTHY SUBJECTS"** is a bonafide record of work done by **Dr. FAZAL ILAHI. J** under our guidance and to our satisfaction, during his postgraduate study period of 2015-2018.

This dissertation is submitted to **THE TAMILNADU Dr. M.G.R. MEDICAL UNIVERSITY** in partial fulfilment for the award of the degree of **MASTER OF DENTAL SURGERY - PERIODONTICS, BRANCH II**. It has not been submitted (partial or full) for the award of any other degree or diploma.

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TITLE OF DISSERTATION	**ASSESSMENT OF PERIOSTIN LEVELS IN GINGIVAL CREVICULAR FLUID OF PATIENTS WITH CHRONIC PERIODONTITIS AND AGGRESSIVE PERIODONTITIS AND COMPARISON WITH THE HEALTHY SUBJECTS**
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DURATION OF THE COURSE	**3 YEARS**
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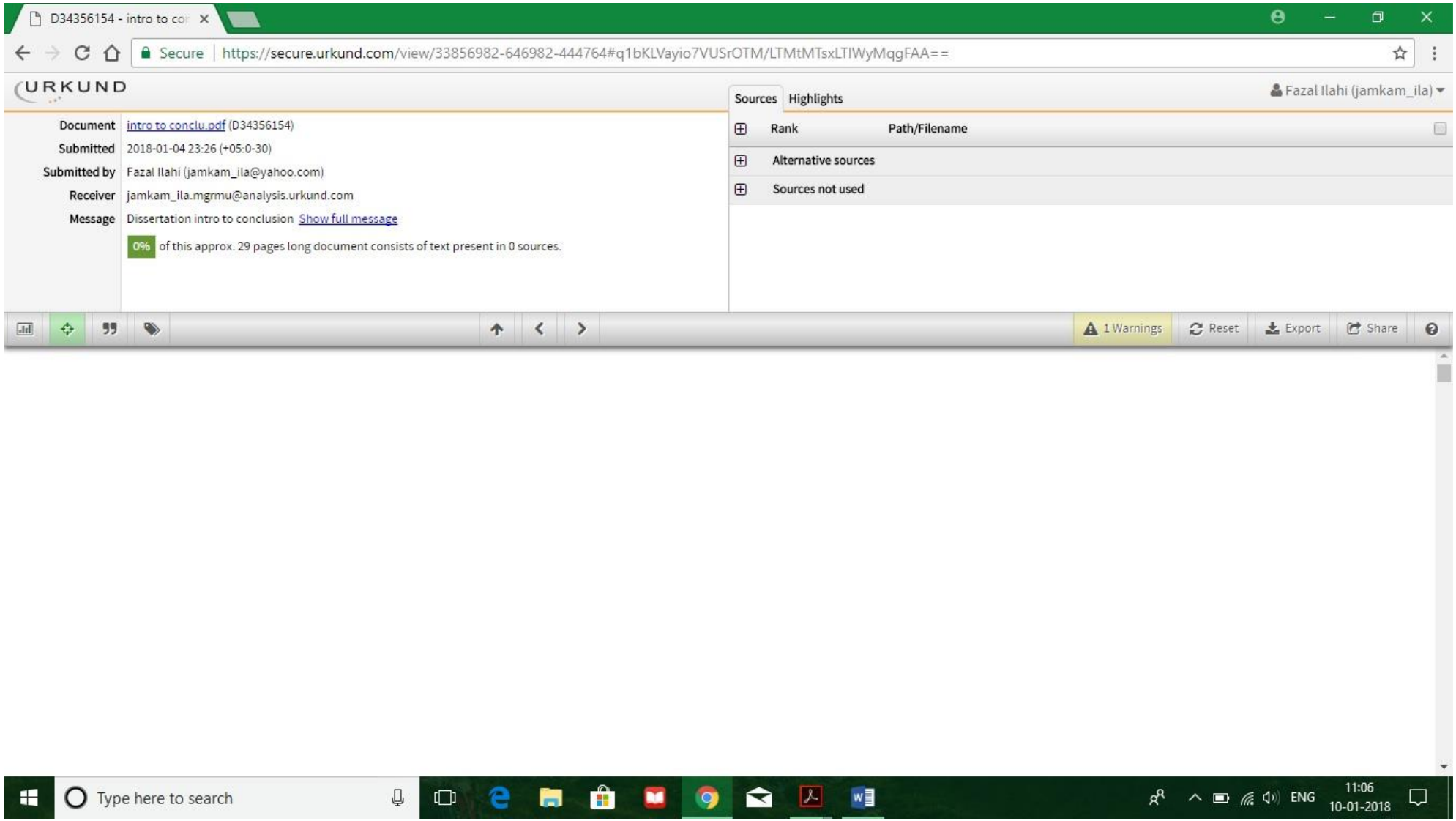
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ABSTRACT

Background: The biological functions of periostin, a matricellular protein, include the importance of it for connective tissue integrity in both health and disease along with regulation in formation of bones. The purpose of this present study was to assess the levels of periostin in gingival crevicular fluid of patients with chronic periodontitis and aggressive periodontitis and to compare them with that of healthy subjects.

Materials and methods: A total of 39 individuals were enrolled in the study. Individuals were divided into three groups following clinical and radiographic examinations: the healthy group (n = 13), the chronic periodontitis group (n = 13) and the aggressive periodontitis group (n = 13). Gingival crevicular fluid samples were collected using microcapillary pipette and periostin levels were determined using the enzyme-linked immunosorbent assay.

Results: The mean levels of total periostin in gingival crevicular fluid were 182.41 pg/µl, 79.87 pg/µl and 49.28 pg/µl for the healthy, chronic periodontitis, aggressive periodontitis groups respectively. There was a statistically significant difference between the groups ($p < 0.05$). The mean levels of total periostin in gingival crevicular fluid were significantly lower in the chronic periodontitis and aggressive periodontitis groups than in the healthy controls. There was a statistically significant difference among healthy and chronic periodontitis groups ($p < 0.05$), among healthy and aggressive periodontitis groups ($p < 0.05$) and also among chronic periodontitis and aggressive periodontitis groups ($p < 0.05$). When all clinical groups were examined together, there were negative correlations between periostin levels in gingival crevicular fluid and age, mSBI, Plaque Index, CAL ($r = -0.303, -0.788, -0.655, -0.691$ respectively).

Conclusion: The periostin levels in gingival crevicular fluid decreased with increased severity of the periodontal disease. When the results in the present study are considered in conjunction with those of previous reports, it might be concluded that the periostin level in gingival crevicular fluid can be considered as a reliable marker in the diagnosis of periodontal diseases and disease activity.

Key words: Gingival crevicular fluid; ELISA; periostin; chronic periodontitis; aggressive periodontitis.

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LIST OF ABBREVIATIONS

AgP	Aggressive periodontitis
bFGF	basic Fibroblast growth factor
BMP	Bone morphogenetic protein
CAL	Clinical attachment loss
CP	Chronic periodontitis
Dpc	Days post-conception
ECM	Extracellular matrix
ELISA	Enzyme linked immunosorbent assay
FAK	Focal adhesion kinase
GCF	Gingival crevicular fluid
Gla	γ- carboxy glutamate
Glu	Glutamic acid residues
hPDLSC	human PDL Stem Cell
IL	Interleukin
LOX	Lysyl oxidase
LPS	Lipopolysaccharides
MMP	Matrix metalloproteinases
MPO	Myelo peroxidase
mSBI	modified Sulcular Bleeding Index
OCN	Osteocalcin
OPN	Osteopontin

Osx	Osterix
PCR	Polymerase chain reaction
PD	Probing depth
PDGF	Platelet derived growth factor
PDL	Periodontal ligament
POSTN	Periostin
rhPOSTN	Recombinant human POSTN
Runx2	Runt related transcription factor 2
TGF	Transforming Growth Factor
TNF	Tumor Necrosis Factor
TRAP	Tartrate resistant acid phosphatase
kDa	KiloDalton
ng/ml	nanogram/milliliter
pg/µl	picogram/microliter

INTRODUCTION

INTRODUCTION

Periodontitis is defined as "an inflammatory disease of the supporting tissues of the teeth caused by specific microorganisms or groups of specific microorganisms, resulting in progressive destruction of the periodontal ligament (PDL) and alveolar bone with increased probing depth formation, recession, or both." [1] Imbalances in the host's immunoinflammatory responses are thought to underlie the pathogenesis of chronic periodontitis (CP) and aggressive periodontitis (AgP).

CP has been defined as "an infectious disease resulting in inflammation within the supporting tissues of the teeth, progressive attachment loss, and bone loss."[2] AgP comprises a group of rare, often severe, rapidly progressive forms of periodontitis, often characterized by an early age of clinical manifestation and with the following characteristics - non-contributory medical history, rapid attachment loss and bone destruction, familial aggregation of cases. [3]

Periodontal disease results from a complex interplay between the subgingival biofilm and the host immune-inflammatory events that develop in the gingival and periodontal tissues in response to the challenge presented by the bacteria. The net result of the inflammatory changes is breakdown of the fibers of the periodontal ligament, resulting in clinical loss of attachment, together with resorption of the alveolar bone. [4]

The inflammatory and immune processes that develop in the periodontal tissues in response to the long-term presence of the subgingival biofilm are protective by intent but result in considerable tissue damage. This has sometimes been referred to as *bystander damage*, denoting that the host response is mainly responsible for the tissue damage that occurs, leading to the clinical signs and symptoms of periodontal disease. This host response is orchestrated by mediators like cytokines, prostanoids,

and matrix metalloproteinases (MMPs). Cytokines play a fundamental role in inflammation, and act as messengers to transmit signals from one cell to another. The prostanoids are the most universally distributed autacoids in the body and are synthesized locally at rates governed by the release of arachidonic acid from membrane lipids in response to appropriate stimuli. MMPs are a family of proteolytic enzymes that degrade extracellular matrix molecules such as collagen, gelatin, and elastin. [5]

Gingival crevicular fluid (GCF) is an inflammatory exudate containing a wide variety of molecules, such as proteins, enzymes, cytokines, mediators, and immunoinflammatory and bacterial cellular elements from peripheral blood and periodontal tissues. Due to the simple, non-invasive nature of its collection, GCF is a tool for observing the pathogenesis of periodontal disease. GCF components have been used to detect periodontal disease activity and to distinguish patients at risk for periodontal disease. [6]

Periostin (POSTN) has been shown to be an important regulator of bone formation. [7] Despite its preliminary description in bone, its biological functions are also essential for connective tissue integrity in both health and disease. [8] POSTN functions as a matricellular protein in cell activation by binding to their receptors on cell surface, thereby exerting its biological activities. [9]

POSTN secreted by fibroblasts, is found to be present in various tissues, serum, saliva and also GCF. GCF POSTN levels have been found to decrease in relation to the progression and severity of CP [10] and also in AgP [11].

Hence, POSTN can act as a novel biomarker with respect to the pathogenesis of CP and AgP. In this present study, the POSTN levels in GCF of CP

and AgP patients are assessed and compared with that of healthy subjects as the previous studies concentrated only on the comparison between its presence in serum, saliva and in gingivitis patients. [10, 11]

AIM AND OBJECTIVES

AIM AND OBJECTIVES

The aim of this study was to investigate POSTN levels in the GCF of patients with CP and AgP and compare them with those of healthy individuals.

The objectives of this study were

- To assess the levels of POSTN in GCF of the healthy subjects and patients with CP and AgP.
- To compare the levels of POSTN in GCF between and among the three groups.
- To find the correlation between the levels of POSTN in GCF and age, Plaque Index, mSBI, CAL in the three groups.

REVIEW OF LITERATURE

Periodontal disease results from a complex interplay between the subgingival biofilm and the host immune-inflammatory events that develop in the gingival and periodontal tissues in response to the challenge presented by the bacteria. The net result of the inflammatory changes is breakdown of the fibers of the periodontal ligament, resulting in clinical loss of attachment, together with resorption of the alveolar bone. [4]

The inflammatory and immune processes that develop in the periodontal tissues in response to the long-term presence of the subgingival biofilm are protective by intent but result in considerable tissue damage. The majority of the tissue damage in periodontitis derives from the excessive and dysregulated production of a variety of inflammatory mediators and destructive enzymes such as, cytokines, prostanoids, and MMPs, in response to the presence of the subgingival plaque bacteria.[5]

Originally known as osteoblast-specific factor, POSTN was first identified in a mouse osteoblastic cell line as a putative cell adhesion protein for preosteoblasts (**Takeshita *et al.*, 1993**). [16] It was then renamed POSTN due to its preferential location in the periosteum. POSTN expression is not restricted to bone as it is predominantly expressed in collagen-rich fibrous connective tissues subjected to constant mechanical stress, such as PDL, [17, 18, 19] heart valves [20] and tendons. [21]

POSTN expression is also increased in a large variety of tumors including colon, bladder, breast, non-small cell lung cancer, head and neck, oral, and pancreas. POSTN up-regulation usually correlates with aggressiveness and/or poor survival of hypoxic tumor cells. [22] The preferential expression of POSTN in collagen rich tissues submitted to mechanical stress such as periosteum from development to perinatal life, as well as up-regulation during fracture healing, suggests that it may play

a crucial role in bone maintenance and regeneration. POSTN has been shown to be an important regulator of bone formation. [7] Despite its preliminary description in bone, its biological functions are also essential for connective tissue integrity in both health and disease. [8]

Structure of POSTN

POSTN gene had been cloned in several species (mouse, rat, chicken, bovine, xenopus, etc.) and is located at locus 13q13.3 in human and 3C in mouse. Originally cloned from both human placental and osteosarcoma tissue, POSTN is highly conserved between human and mouse with 89.2% homology. The mouse POSTN cDNA is 3,187 bp long and contains an 18-bp 5′ untranslated region, a 733-bp 3′ untranslated region, and an open reading frame of 2,436 bp corresponding to a protein precursor of 838 amino acids (Fig. 1).

The protein is composed of a signal sequence, followed by an EMI domain rich in cysteine, 4 repeated and conserved FAS- 1 domains, and a C-terminal hydrophilic and variable domain. Alternative splicing of the C-terminal domain gives rise to at least five different human isoforms (**Takeshita *et al.*, 1993, Hoersch S and Andrade-Navarro MA, 2010**). [16, 23]

An additional variability caused by single nucleotide polymorphism was also reported by National Center for Biotechnology Information (NCBI database). Due to the presence of FAS-1 domains, POSTN belongs to the fasciclin family and shows a structure similar to the insect axon guidance fasciclin and transforming growth factor (TGF)-β-inducible protein involved in cell attachment (**Thapa *et al.*, 2007**). [24]

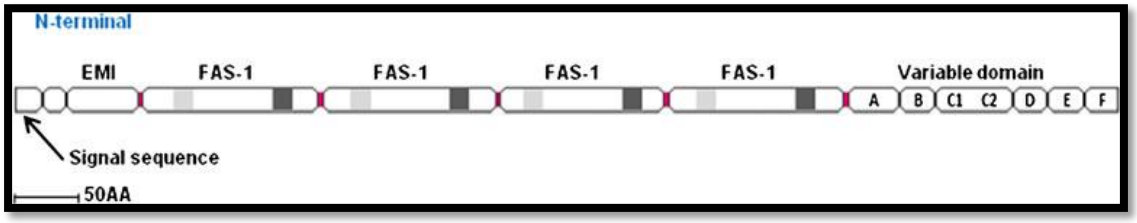


Fig. 1: Structure of periostin. POSTN protein sequence showing the signal sequence, the EMI domain, the four FAS-1 domains, and the C-terminal variable region with the six different cassettes (A–F) whose combination gives rise to different isoforms. Each FAS-1 domain contains an N-terminal recognition site for the γ-glutamyl carboxylase and a cell adhesion site. [7]

The presence of integrin binding motifs in the second and fourth FAS-1 domains suggests that POSTN is implicated in cell adhesion, as these domains have been shown to mediate TGF-β-inducible protein adhesion to α3β1. [23] In addition, each FAS-1 domain is rich in glutamate residues and contains an N-terminal recognition site for the vitamin K-dependent enzyme γ-glutamyl carboxylase (γ-carboxylase recognition sites, or CRS) responsible for the post-translational modification of glutamic residues (Glu) to γ-carboxyglutamate (Gla).

POSTN also possesses four putative N-glycosylation sites and a heparin-binding domain (arginine-rich consensus sequence) at its C-terminal end creating a potential binding site for glycoproteins, glycosaminoglycans, and proteoglycans. [18, 25] Finally, POSTN can also form disulfide-bonded dimers through its EMI domains. [26, 27] The POSTN isoforms seem to be differentially expressed during development or following stress such as infarct or neoplasm. [19, 28, 29, 20, 31] Alternative splicing of POSTN gives rise to different isoforms, specific of a tissue type, a

development stage or a disease. The C-terminal part of POSTN is encoded by exons 15 to 21, which present themselves as six cassette exons, a to f, according to the denomination established by **Horiuchi *et al.*** in **1999**. [18] These cassettes can be presented or deleted in mature mRNA in various combinations, giving rise to different isoforms. [18] POSTN isoform 1 contains the six cassettes, whereas in isoform 3, also known as POSTN-like factor, cassette e is missing. [29] Additionally, a variant lacking both b and e cassettes had been described to be preferentially expressed in the periosteum and PDL as well as in heart tissues after myocardial infarction. [32] The predicted amino acid sequence analysis of the C-terminal end identified a nuclear localization sequence implying that some isoforms will be localized in the nucleus, as it has been shown for isoform 3. [28, 29] Even though this C-terminal hydrophilic region is devoid of known protein domains, specific roles of certain isoforms have been reported in *in vitro* cell invasiveness according to their differential expression patterns (**Kim *et al.*, 2008**, **Kim *et al.*, 2005**).[31, 33] The fact that POSTN, which has been localized in the nucleus and the cytosol of cells also bears a signal sequence and is secreted, suggests that POSTN isoforms can have intracellular and extracellular functions (**Kudo, 2011**). [32] Finally, a tri-dimensional structural model for POSTN has been proposed: the four FAS domains of human POSTN have a secondary structure consisting of a helix-turn-helix motif, while the C - terminal region presents beta-strands. [16, 23, 34]

POSTN expression in bone and other tissues

POSTN is preferentially expressed in the periosteum, which covers a large majority of bones, and in the PDL of the teeth, which is the soft, richly vascular and cellular connective tissue which surrounds the roots of the teeth and joins the root

cementum with the socket wall. Periosteum is responsible for changes in bone diameter and cortical thickness, and bone size is related to bone strength.

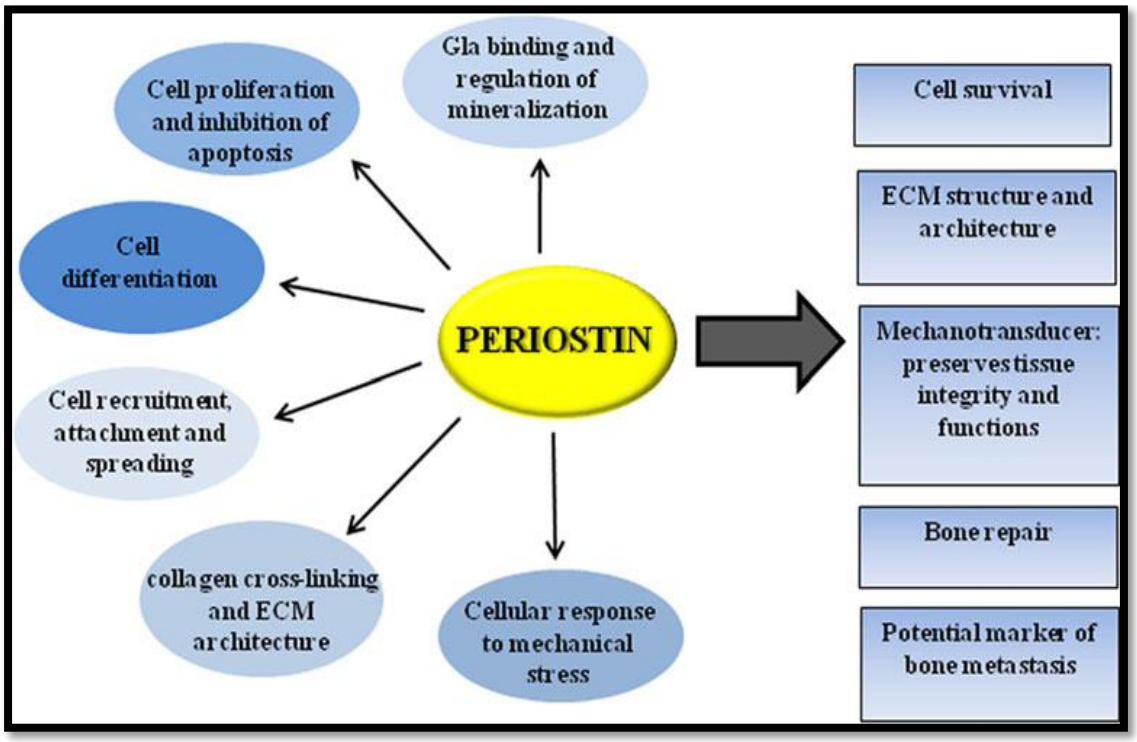


Fig. 2: Putative functions of periostin in bone. POSTN is involved in several cellular and extracellular functions resulting in stimulation of bone formation during development, response to mechanical stress, or bone fracture. Overexpressed in tumors, POSTN may be a potential marker of bone metastasis. [7]

Periosteal apposition occurs throughout life: it is high during growth and decreases in adult life. Beyond its crucial importance during embryogenesis, periosteum is largely involved in fracture healing. [35] In a study by **Kruzynska-Frejtag *et al*.** in **2004** study, during embryogenesis, POSTN mRNA was detected as early as 9.5 days post-conception (dpc) in mouse embryos. In developing bone, at least four protein isoforms could be detected from 12 to 19.5 dpc with an expression more or less

important over time. [19, 29] Fetal development of the skeleton implied two processes: intramembranous ossification involved mesenchymal stem cells, which developed into osteoblasts, whereas in endochondral ossification, a cartilage template first formed by chondrocytes was then replaced by bone. In the developing long bone, immunolocalization showed that POSTN isoforms 2 and 3 were localized to proliferating and hypertrophied chondrocytes from 12.5 dpc and all along the differentiation process. At 16.5 dpc, isoform 3 expression appeared in mesenchymal preosteoblasts cells from the periosteum and in ameloblasts and odontoblasts of developing teeth, whereas isoform 2 was primarily localized in the mesenchymal cells of the perichondrium till 19.5 dpc. [19, 28]

Additionally, isoform 3 showed both nuclear and cytoplasmic expressions in accordance with the presence of a nuclear localization sequence, whereas isoform 2 was only found in cytoplasm. [29] However, as the 2 antibodies used in this study have been raised against a peptide arising from cassette b or e to recognize isoform 3 or isoform 2, respectively, they should, in principle, also detect all the isoforms bearing one of these cassettes. Additionally, they would miss isoforms lacking cassettes b and e, described to be expressed in periosteum and PDL.[28, 32]

Overall, these data showed that there was a spatial and temporal expression and localization of POSTN isoforms, suggesting different roles for these variant proteins during osteogenesis. As in embryonic bone, multiple protein variants of POSTN were expressed, but to a lesser extent, in neonates, young, and adult rodents bone. [18, 26, 29, 36]

POSTN protein (at least isoform 3) was detected in mesenchymal cells and preosteoblasts and osteoblasts of the periosteum and in the osteoblasts lining

trabecular bone. [18, 36, 37] Similarly, immunohistochemistry on human bone with an antibody recognizing the N-terminal part of POSTN common to all variants revealed that POSTN was largely expressed in the ECM of the cambial layer of the periosteum of long bones and calvaria. POSTN mRNA was detected in periosteum cells but not in osteoblasts, osteocytes, or lining cell of the underlying bone. [38] By contrast, using two antibodies recognizing isoform 2 or 3, one group showed that these isoforms were not expressed in the periosteum nor epiphyseal plate of adult normal rat bone. [39] However, expression of both POSTN variants was induced by chronic overload in the cellular periosteum, articular cartilage, osteoblasts, osteoclasts, and osteocytes in a time and spatial localization pattern. [36, 39] As mentioned earlier, these antibodies might detect more than one isoform and miss variants lacking b and e cassettes. Furthermore, in the periosteum as in the infarcted myocardium, POSTN would be secreted, at least in part, as a cleaved form lacking its C-terminal domain. [26]

Overall, these data suggested that POSTN was preferentially expressed in the periosteum at a high level during embryogenesis and bone growth. In adult, it was re-expressed after mechanical stress and fracture where bone formation was important. The concomitant stimulation of POSTN and inhibition of mineralization by activin A and the presence of ectopic mineral deposit on bone in POSTN knockout mice strengthened the association of POSTN with the early stages of osteoblast differentiation and bone formation. [26, 40]

It was found in the study by **Taniguchi *et al.* (2014)** that POSTN regulated proliferation and differentiation of keratinocytes in the epithelial–mesenchymal interactions using a three-dimensional organotypic air–liquid interface co-culture system. The release of interleukin (IL)-1α from keratinocytes and

subsequent IL-6 production from fibroblasts were critical for keratinocyte proliferation and differentiation. POSTN secreted from fibroblasts was required for IL-1α-induced IL-6 production and enhanced IL-6 production by activation of the NF-kB pathway synergistically with IL-1α. Thus, the combination of an autocrine loop of POSTN and a paracrine loop composed of IL-1α and IL-6 regulated keratinocyte proliferation and differentiation in the epithelial–mesenchymal interactions, and POSTN tuned the magnitude of keratinocyte proliferation and differentiation by interacting with the paracrine IL-1α/IL-6 loop. [42]

POSTN expression in teeth

Wilde *et al*. in **2003** examined the expression of POSTN mRNA during experimental tooth movement. Experimental tooth movement was achieved in 7-week-old male Sprague-Dawley rats. In control specimens without tooth movement, the expression of POSTN mRNA was uniformly observed in the PDL surrounding the mesial and distal roots of the upper molars and was weak in the PDL of the root furcation area. The POSTN mRNA-expressing cells were mainly fibroblastic cells in the PDL and osteoblastic cells on the alveolar bone surfaces. The divergent expression of POSTN mRNA in the PDL began to be observed at 3 h and continued up to 96 h after tooth movement. The maximum changes, which showed stronger staining in the pressure sites than in the tension sites, were observed at 24 h. The expression of POSTN mRNA in the PDL 168 h after tooth movement exhibited a similar distribution to that of the control specimens. These results suggested that POSTN was one of the local contributing factors in bone and periodontal tissue remodeling following mechanical stress during experimental tooth movement. [45]

In a study by **Kruzynska-Frejtag *et al.*** in **2004**, POSTN mRNA and protein expression were analyzed throughout normal tooth development (embryonic day 9.5–new born) and within both *Bmp4*- and *Msx2*-null embryos. POSTN mRNA was initially present within the embryonic day 9.5 first branchial arch epithelium and then shifts to underlying ectomesenchyme. Both mRNA and protein were asymmetrically localized to the lingual/palatal and buccal side during the early epithelial–mesenchymal interactions. POSTN was also found to be present in dental papilla cells and within the trans-differentiating odontoblasts during the bell and hard tissue formation stages of tooth development. [19]

In situ hybridization and immunohistochemistry showed that POSTN was expressed in the PDL, in dental pulp at the sites of hard/soft tissue interfaces in mouse and human tooth. In a study by **Afanador *et al.*** in **2005**, unilateral maxillary tooth extraction was performed in 3-week-old male mice to produce occlusal hypofunction of the right mandibular molars. The expression of POSTN was examined by real time-polymerase chain reaction (PCR) and in situ hybridization at 12, 24, 72 and 168 h after the tooth extraction. The real time-PCR analysis showed that POSTN significantly decreased at 24 h to 14.5% of those in control group. But the recovery began at 72 and 168 h and no significant difference was observed. As determined by in situ hybridization analysis, the number of POSTN expressing PDL cells showed a marked decrease at 24 h, although an increase was observed from 72 h until the distribution was almost similar to that of the control group at 168 h. [43]

Wen *et al.* in **2010** investigated whether POSTN was expressed in the human PDL *in situ* and the mechanisms regulating POSTN expression in PDL fibroblasts *in vitro.* With immunohistochemistry, POSTN was identified in the PDL,

with expression lower in teeth with reduced occlusal loading. *In vitro* application of uniaxial cyclic strain to PDL fibroblasts elevated POSTN mRNA levels, depending on the age of the patient. Treatment with TGF-β1 also significantly increased POSTN mRNA levels, an effect attenuated by focal adhesion kinase (FAK) inhibition. FAK-null fibroblasts contained no detectable POSTN mRNA, even after stimulation with cyclic strain. They concluded that POSTN was strongly expressed in the human PDL. *In vitro,* POSTN mRNA levels were modulated by cyclic strain as well as TGF-β1 *via* FAK-dependent pathways. [44]

POSTN is secreted by fibroblasts and osteoblasts in PDL. As in bone, it was noticeably absent from terminally differentiated mineralized tissues (dentin, alveolar bone). In control specimens, the expression of POSTN mRNA was uniformly observed in PDL fibroblastic cells in the sections stained with antisense probe. In addition, a number of gingival fibroblasts between the first and second molars and osteoblastic cells on alveolar bone surfaces also expressed POSTN mRNA. No positive reaction was observed in dentin, cementum, dental pulp, and alveolar bone.[43]

The study by **Choi *et al***. in **2011** included 45 Wistar male rats (12 weeks of age) whose upper-right first molars were relieved from occlusion for 24 hours, 72 hours, 7 days or 21 days. The PDL was examined histologically, and changes in the gene and protein levels of POSTN and connective tissue growth factor were investigated. The PDL space width was reduced significantly. Histologically, an initial reduction in the fiber number and thinning of PDL fibers were observed, followed by disarrangement of the PDL fibers and their attachments to the alveolar bone; finally, the PDL fibers lost their meshwork structure. Real-time PCR results revealed sharp down-regulation of the POSTN and connective tissue growth factor mRNA levels at 24

hours and 72 hours, respectively, which continued throughout the experiment. Immunohistochemical analysis revealed that POSTN localized to both the cellular elements and the extracellular matrix, whereas connective tissue growth factor localized only to the cellular elements. POSTN and connective tissue growth factor immunoreactivities became very weak without masticatory load. [46]

Padial-Molina M *et al.* in **2013** exposed human PDL cultures to inflammatory mediators (tumor necrosis factor (TNF)-α), bacterial virulence factors (Porphyromonas gingivalis liposachharides (LPS)) or a combination in a biomechanically challenged environment. Culture conditions were applied for 24 hours, 4 days, and 7 days. POSTN and TGF-ß inducible gene clone H3 mRNA expression from cell lysates were analysed. POSTN and TGF-ß inducible gene clone H3 proteins were also detected and semi-quantified in both cell lysates and cell culture supernatants by Western blot. In addition, POSTN localization by immunofluorescence was performed. In a mechanically challenged environment, POSTN protein was more efficiently incorporated into the matrix compared to the non-loaded controls. Interestingly, chronic exposure to pro-inflammatory cytokines and/or microbial virulence factors significantly decreased POSTN protein levels in the loaded cultures. There was greater variability on TGF-ß inducible gene clone H3 levels, and no particular pattern was clearly evident. Thus, inflammatory mediators TNF-α and bacterial virulence factors (Porphyromonas gingivalis LPS) decreased POSTN expression in human PDL fibroblasts. [47]

In a study by **Padial-Molina M *et al.*** in **2014**, human PDL cells were cultured under different conditions: control, POSTN (50 or 100 nanogram/millilitre(ng/ml)), and fibroblast growth factor 2 (10 ng/ml) to evaluate cell

proliferation (by Ki67), cell migration (by scratch assays) and PI3K/AKT/mTOR pathway activation (by Western blot analyses of total AKT, phospho-AKT and PS6). A different set of cultures was challenged by adding TNF α (10 ng/ml) and Porphyromonas gingivalis LPS (200 ng/ml) to evaluate the effects of POSTN. POSTN significantly increased cell proliferation (two-fold), migration (especially at earlier time points and low dose) and activation of survival signalling pathway (higher phosphorylation of AKT and PS6). Furthermore, POSTN promoted similar cellular effects even after being challenged with pro-inflammatory cytokines and bacterial virulence factors. It was concluded that POSTN acted as an important modulator of human PDL cell–matrix dynamics, modulated human PDL proliferation, migration and PI3K/AKT/mTOR pathway and also helped in overcoming the altered biological phenotype that chronic exposure to periodontal pathogens and pro-inflammatory cytokines produce in human PDL cells. [48]

Cobo *et al.* in **2015** used Western blot and double immunofluorescence coupled to laser-confocal microscopy to investigate the occurrence and distribution of POSTN in different segments of the human gingiva in healthy subjects. By Western blot, a protein band with an estimated molecular mass of 94 kiloDalton (kDa) was observed. POSTN was localized at the epithelial-connective tissue junction, or among the fibers of the periodontal ligament, and never co-localized with cytokeratin or vimentin thus suggesting it as an extracellular protein. These results demonstrated the occurrence of POSTN in adult human gingiva; its localization suggested a role in the bidirectional interactions between the connective tissue and the epithelial cells, and therefore in the physio-pathological conditions in which these interactions were altered. [49]

Liu and Chen in **2017** studied inhibition of hypoxia-induced apoptosis in human PDL fibroblasts by POSTN. The apoptosis rate of periodontal ligament fibroblasts was increased under hypoxic conditions. The protein expression levels of hypoxia-inducible factor-1α and Bcl-2/E1B 19 kDa interacting protein 3 were upregulated in periodontal ligament fibroblasts under hypoxic conditions. Overexpression of POSTN decreased hypoxia-induced apoptosis, as well as hypoxia-inducible factor-1α and Bcl-2/E1B 19 kDa interacting protein 3 level. The phosphorylation level of Akt/PKB on Ser473 was increased in periodontal ligament fibroblasts transfected with POSTN. POSTN activated the Akt/PKB signalling pathway through the αVβ3 integrins pathway. The addition of an Akt/PKB inhibitor resulted in an increase in hypoxia-induced apoptosis compared with the control group. Moreover, the protective effect of POSTN was disrupted in PDL fibroblasts co-treated with Akt/PKB inhibitor. Under hypoxic conditions, POSTN decreased hypoxia-inducible factor-1α and Bcl-2/E1B 19 kDa interacting protein 3 expression levels, and inhibited apoptosis in human PDL fibroblasts via activating the Akt/PKB kinase signalling, which provided a novel insight into the regulation of apoptosis in periodontal diseases. [50]

In **Wu *et al.*'s (2017)** study, the human periodontal ligament mesenchymal stem cells (hPDLSCs) were isolated and characterized by their expression of the cell surface markers CD44, CD90, CD105, CD34, and CD45. Next, 100 ng/ml recombinant human POSTN protein (rhPOSTN) was used to stimulate the hPDLSCs. Lentiviral POSTN shRNA was used to knockdown POSTN. The cell counting kit-8 and scratch assay were used to analyze cell proliferation and migration, respectively. Osteogenic differentiation was investigated using an alkaline phosphatase

activity assay, alizarin staining, and quantitative calcium analysis and related genes/protein expression assays. Isolated hPDLSCs were positive for CD44, CD90, and CD105; and negative for CD34 and CD45. In addition, 100 ng/ml rhPOSTN significantly accelerated scratch closure, and POSTN-knockdown cells presented slower closure at 24 h and 48 h. Furthermore, the integrin inhibitor, Cilengitide depressed the scratch closure that was enhanced by POSTN at 24 h. The CCK8 assay showed that 100 ng/ml rhPOSTN promoted hPDLSC proliferation. Moreover, 100 ng/ml rhPOSTN increased the expression of Runt related transcription factor 2 (Runx2), osterix (Osx), osteopontin (OPN), osteocalcin (OCN), and vascular endothelial growth factor, and enhanced alkaline phosphatase activity and mineralization. POSTN silencing decreased the expression of Runx2, Osx, OPN, OCN, and vascular endothelial growth factor, and inhibited alkaline phosphatase activity and mineralization. [51]

Transcriptional regulation of POSTN expression

A number of studies, especially in cancer in which POSTN was largely found to be implicated, had shown that many factors can modify POSTN expression. Among the transcription factors involved in osteoblast differentiation, Runx2/cbfa1, Wnt/β-catenin, and Osx pathways are essential in the commitment of pluripotent mesenchymal cells to the osteoblastic lineage. [52] In MC3T3-E1 osteoblast-like cells overexpressing Runx2, a factor involved in the commitment of stem cells to preosteoblasts, POSTN was positively regulated, suggesting its involvement in early bone cell differentiation.[53] Wnt-3 pathway was shown to downregulate POSTN expression in an epithelial cell model. Infection of cells with Wnt-3 and inhibition of glycogen-synthase kinase-3β regulate a set of genes that include cyclooxygenase-2 and

POSTN. **Haertel-Wiesmann M *et al.* (2000)** compared gene profiles regulated by Wnt-3, β-catenin, and inhibition of glycogen-synthase kinase-3β. Cyclooxygenase-2 was up-regulated and POSTN was ten-fold down-regulated by both Wnt-3 and inhibition of glycogen-synthase kinase-3β. [54]

Twist-1 is another transcription factor that has been shown to play both positive and negative roles in cell differentiation. Twist-1 and POSTN were co-expressed by differentiating osteoblasts and fibroblasts at the osteogenic front of calvaria and at the alveolar bone surfaces in PDL. The occlusal force might have putative roles in POSTN and Twist gene expression in the PDL and the changes in their expression level during hypofunction might be considered a form of adaptation to environmental changes.[43, 55, 56]

c-Fos/AP-1 is a transcription factor that plays an important role in bone cell proliferation and differentiation. [57] Human bone tissues from patients with fibrous dysplasia, associated with increased expression of c-Fos, expressed high levels of POSTN. [38] Similarly, transgenic mice overexpressing c-Fos develop sclerotic lesions with transformed osteoblasts expressing high levels of POSTN, whereas normal osteoblasts did not, suggesting that c-Fos pathway might represent one mechanism for POSTN up-regulation, at least in pathological conditions resulting in altered collagen fibrillogenesis and deposition. Two potential binding sites for c-Fos/AP1 had been described in the POSTN promoter. [38]

Regulation of POSTN expression by hormones

The estrogens inhibited periosteal bone growth while being anabolic on endosteal and trabecular bone. [62] **Ogita M *et al.* (2008)** showed that estrogens promoted undifferentiated periosteal cell proliferation but inhibited their differentiation in young

rodents by attenuating parathyroid hormone or bone morphogenetic protein (BMP) -2-induced actions suggesting a possible different regulation of POSTN. The parathyroid hormone promoted and subsequently suppressed proliferation of periosteal osteoblast progenitors *in vitro* and *in vivo*. Both parathyroid hormone and estrogen seemed also to protect undifferentiated periosteal cells from apoptosis. [58]

In human PDL cell culture, estrogens were reported to enhance cell proliferation and POSTN mRNA expression through binding to estrogen receptor beta, but also to stimulate osteoblastic differentiation by increasing alkaline phosphatase activity, osteocalcin production, and mineralized nodules formation. In **Mamalis *et al.*'s (2011)** study, the short interfering RNA technique was used to inhibit estrogen receptor beta expression in human PDL cells. Human PDL cells were isolated and fully characterized. A colorimetric assay was applied for the determination of alkaline phosphatase. An ELISA kit was used to detect osteocalcin levels. Collagen synthesis was determined by measuring the incorporation of L-[3H] praline. Real time-PCR was performed for detection of POSTN mRNA relative gene expression. Estrogen receptor beta mRNA was expressed in human PDL cells and significant inhibition of mRNA expression and estrogen receptor beta mature protein was evident in the short interfering RNA group. At 72 h, there was a significant increase in non-transfected human PDL cell proliferation after estradiol stimulation. Addition of 17b-estradiol significantly enhanced alkaline phosphatase activity and production of osteocalcin in non-transfected cells but had no effect on collagen synthesis. A clear increase in POSTN mRNA expression levels was observed after incubating human PDL cells with estradiol. In human PDL - short interfering estrogen receptor beta cells, the application of estradiol did not produce any evident differences in POSTN mRNA expression. [61]

Regulation of POSTN expression by growth factors

Among growth factors and cytokines, POSTN expression was shown to be regulated by several members of the TGF-β superfamily. TGF-β and BMP are well-known regulators of bone development by stimulating the differentiation of osteoblast progenitors (**Janssens *et al.*, 2005, Canalis *et al.*, 2005**). [63, 64] TGF-β, BMP-2, activin, and retinoic acid all stimulated POSTN expression in osteoblasts. [18, 40, 44, 65, 67] Blocking TGF-β receptor activation or the non-canonical FAK pathway reduced POSTN mRNA expression. Additionally, FAK/src inhibition in human PDL fibroblasts reduced nuclear translocation of twist and POSTN mRNA levels. [44] These data demonstrated that TGF-β could regulate POSTN expression through activation of TGF-β receptors but also through FAK/src signalling pathway. FAK activated signalling molecules, leading to twist-1 translocation to the nucleus and subsequent POSTN activation. [44, 56] The fact that activin and TGF-β inhibited mineralization and that POSTN expression was negatively regulated as mineralization proceeds suggested that TGF-β and activin might control the onset of mineralization, at least in part, via the up-regulation of POSTN expression (**Eijken *et al.*, 2007**).[40]

POSTN was shown to be up-regulated by platelet derived growth factor (PDGF), basic fibroblast growth factors (bFGF), and angiotensin II in cancer cell lines, through different pathways including RTK/PI3K, Ras/MEK, and Ras/p38MAPK (**Li *et al.*, 2004, Li *et al.*, 2006, Erkan *et al.*, 2007, Ouyang *et al.*, 2009**). [66, 67, 68, 69] PDGF and FGF had been shown to stimulate bone formation in vivo, and it could be speculated that they also acted, at least in part, through POSTN regulation (**Kim *et al.*, 2007**). [70] TNF-α increased POSTN expression levels by osteoblasts from adult rat bones submitted to chronic overload (**Rani *et al.*, 2010**). [71] All these mechanisms were

particularly important in POSTN-induced response to environmental stress (i.e., mechanical loading and hypoxia) in order to maintain cell survival and functions in bone (**Li *et al.*, 2006, Erkan *et al.*, 2007, Ouyang *et al.*, 2009, Rios *et al.*, 2008, Ma *et al.*, 2009**). [67, 68, 69, 72, 73]

Increased POSTN expression in response to an environmental stress might represent an adaptative cell strategy to maintain cell survival. However, IL-4 and IL-13, two anti-inflammatory cytokines that inhibited osteoblast proliferation but enhanced their differentiation and matrix production, had been shown to induce POSTN in an *in vivo* mouse model of fibrosis (**Takayama *et al.*, 2006**). [27]

Besides its function in the formation of hydroxyapatite, inorganic phosphate could affect cell functions and gene expression. Indeed, elevation of phosphate as mineralization begin led to a down-regulation of POSTN gene expression *in vitro*, strengthening the association of POSTN with the early stages of osteoblast differentiation and bone formation. [40, 41] All these data demonstrated that POSTN was regulated by factors acting on preosteoblasts rather than on fully differentiated cells and underlined its importance in cell proliferation and early stages of osteoblast differentiation. (Table 1)

Promotion of osteoblast adhesion, differentiation, and survival by POSTN

In MC3T3 and primary rat osteoblasts, POSTN inhibition abolished cell proliferation and differentiation and reduced the expression of cbfa1. [29, 71] POSTN was found to be expressed early in osteoblast and involved in the differentiation process, possibly through integrin binding. Studies on cardiac and cancer cells had extensively reported that POSTN could bind through its FAS-1 domains to the integrins αVβ3, αVβ5, and α6β4 and enhance cell proliferation and survival, migration, and metastasis.

Table 1: Factors involved in positive or negative regulation of POSTN expression [7]

Regulation	Positive	Negative
Transcription factors	Twist	Wnt
	Runx-2	
	c-Fos/AP1	
Hormones, cytokines, and growth factors	Parathormone	Leptin
	Estrogens	
	TGF-β	
	Activin	
	BMP-2	
	Retinoic acid	
	IL-4	
	IL-13	
	TNF α	
	PDGF	
	FGF	
	Angiotensin	
Physicochemical factors	Mechanical stress	Phosphate
	Hypoxia	Microgravity

(**Butcher *et al.*, 2007, Baril *et al.*, 2007, Li *et al.*, 2010, Bao *et al.*, 2004**). [74, 75, 76, 77] However, osteoblasts and osteoclasts expressed different integrins, at least αVβ3 and αVβ5, which might mediate POSTN signalling in bone. POSTN and αVβ3 were concomitantly expressed in human bone tissue, suggesting that POSTN could recruit and attach osteoblasts to bone matrix. Downstream effectors of POSTN binding to integrins included FAK, Rho/PI3-kinase, and Akt/PKB signalling pathways, which induced migration, proliferation, and matrix formation.[68, 74, 76, 77, 78, 79] In bone, POSTN binding to αVβ3 might also activate the downstream FAK and Akt/PKB pathway, one of the most potent pro-survival signalling pathways that had been demonstrated in UMR-106 osteoblast-like cells, to regulate cell migration and survival (**Kashima *et al.*, 2009, Bao *et al.*, 2004, Bonnet *et al.*, 2009**). [38, 77, 80]

By binding to Notch, POSTN up-regulated expression of Notch and Bcl-xl, leading to inhibition of cell death, especially in stress conditions. Mechanical loading experiments on mouse bone had shown that POSTN was upregulated, while sclerostin, a potent antagonist of bone formation through inhibition of Wnt and BMP signalling, was down-regulated.[59, 60, 80, 81] Under mechanical stress, it was possible that POSTN regulates sclerostin secretion by osteocytes through integrin signalling, to stimulate osteoblast activity and maintain bone integrity. [80, 82] The fact that POSTN null mice had their bone biomechanical properties restored by injection of a sclerostin-blocking antibody further lent to support this hypothesis. [80] Alternatively, POSTN might stimulate bone formation directly through Wnt / β-catenin signalling. [83, 84]

In summary, these data showed that POSTN being a pro-survival protein, largely involved in cell response to environmental stress. [73, 75, 85] By binding to integrins or cell surface receptors, POSTN could activate intracellular signalling

pathways leading to inhibition of apoptosis through inactivation of caspases 3 and 9 and pro-survival signalling resulting in increased bone formation. [22, 73, 79, 86]

Involvement of POSTN in ECM structure and organization

POSTN belongs to the matricellular proteins family, that is, proteins that regulate cell functions and cell–matrix interactions rather than contributing directly to the formation of structural elements. [87, 88] In addition to POSTN, this family includes thrombospondin-1, osteonectin, OPN, tenascin-C, and tenascin-X. A common feature of these proteins is that several animal models in which a matricellular protein has been knocked out survive embryogenesis and show only mild phenotypes at birth, consistent with their partial contribution to structural integrity. However, these proteins still play a critical role in collagen assembly, particularly during wound healing. Interestingly and unlike many other matricellular proteins, POSTN is also present in adult animal in collagen-rich tissues such as PDL and bone, suggesting a key role in connective tissue homeostasis.

Inside the cell, POSTN interacted with BMP-1 to increase its deposition in the fibronectin matrix in close proximity of pro-lysyl oxidase (LOX). BMP-1 then induced the proteolytic activation of pro- LOX and promoted collagen cross-linking as assessed by the measurement of pyridinoline and deoxypyridinoline content in collagen. [32, 89] (Fig. 3)

These data demonstrated that intracellular POSTN could act as a scaffold that increased the deposition of BMP-1 into the fibronectin matrix, to activate pro-LOX and promote collagen cross-linking. [32] The overexpression of POSTN in rats increased bone formation and bone mass, but also cardiac tissue viscosity, a measure of collagen cross-linking. [36] Additionally, POSTN had been shown to bind to collagen

type V. [27] Because BMP-1 is involved in the activation—via proteolytic maturation—of other extracellular proteins present in bone including different types of collagens, laminin 5, or probiglycan, these findings suggested that POSTN might have a wider role in matrix organization. [89]

Overall, these data implied that, by its involvement in collagen fibril assembly and cross-linking, POSTN could be essential for proper collagen folding and maintenance of mechanical properties of collagen-rich tissues like bones.

POSTN binds tenascin-C that is expressed in bone and myocardium and inducible by mechanical stimulation, tenascin-C forms a disulfide-linked hexamer called "hexabrachion". [26, 27, 90, 91] POSTN null mice showed an inhibition of the deposition of tenascin-C and a disorganization of the ECM at the lower tibia similar to the phenotype observed in the periostitis in tenascin-C null mice. [26] POSTN and tenascin-C presented similarities in their expression profiles and were both induced by mechanical stress, implying that these two proteins are essential in the extracellular architecture of periosteum.[88, 91] Indeed, co-immunoprecipitation assays demonstrated that tenascin interacts with the FAS-1 domains of POSTN lacking its C-terminal domain but not with intact POSTN, suggesting that the C-terminal domain prevents aggregation of POSTN with tenascin in the endoplasmic reticulum. [26, 27] POSTN was found to be secreted as a cleaved form in the periosteum and it might support the incorporation of tenascin-C hexabrachions in the ECM by binding to tenascin and other proteins like fibronectin. This increased bifurcations of the matrix fibrils to support the meshwork architecture of the ECM. [26, 32]

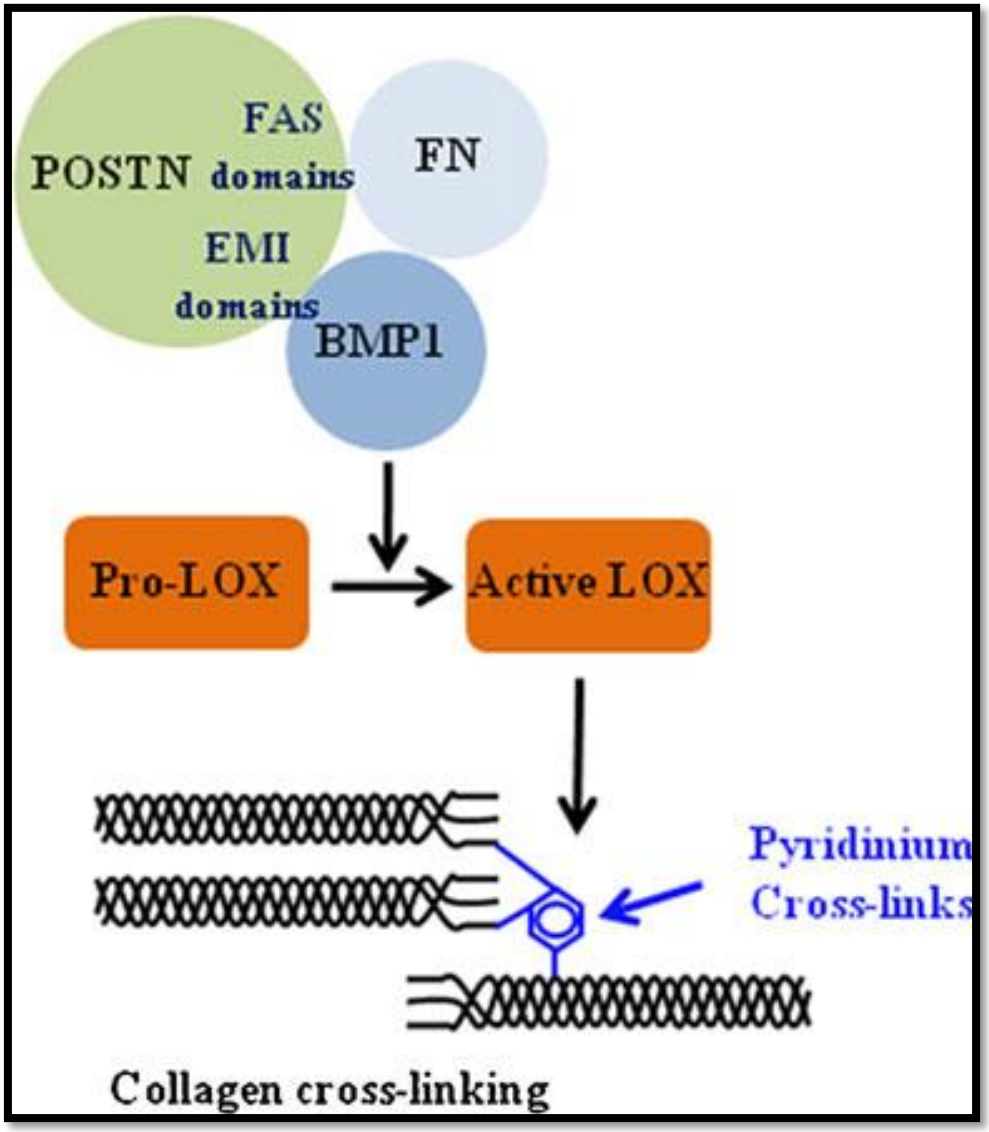


Fig. 3: Periostin is a key regulator of collagen cross-linking. POSTN binds fibronectin (FN) through its EMI domain and to BMP-1 through its FAS-1 domain, promoting the deposition of BMP-1 into the matrix. BMP-1 activates LOX precursor (pro-LOX) to mature active form (LOX). LOX is responsible for the synthesis of pyridinium cross-links, further linking collagen fibers. [7]

However, in situ proximity ligation assays showed that both POSTN and tenascin co-localized in the periphery or inside the cell, indicating a possible interaction of both proteins intracellularly or in the matricellular space. As suggested by **Kudo** in **2011** [32], it was conceivable that POSTN anchored tenascin in the intracellular network with fibronectin and BMP-1 to further induce collagen cross-linking. Additionally, POSTN also possesses a heparin-binding domain at its C-terminal end creating a potential binding site for glycoproteins, glycosaminoglycans, and proteoglycans. [25] Finally, POSTN can also form disulfide-bonded dimers through its EMI domains. [26, 27] (Table 2).

All these data clearly demonstrated that POSTN might support intracellular and extracellular functions, with cellular or secreted isoforms that are differentially expressed. By binding to endogenous fibronectin, tenascin, and BMP-1, cellular POSTN is a key element in collagen cross-linking. [89]

However, all these proteins are also expressed into the ECM, suggesting that POSTN might be involved in extracellular collagen fibrillogenesis and meshwork architecture to support mechanical strength in periosteum. POSTN associates with other matricellular proteins such as connective tissue growth factor, cystein rich protein, and nephroblastoma overexpressed gene family members and thrombospondin known to be involved in some aspect of osteogenesis or chondrogenesis. [32] Furthermore, POSTN had been shown to co-localize with laminin and fibronectin at the basement membrane in hair follicles, corroborating its central role in ECM organization. [92]

Response of POSTN to mechanical stress

Physical activity has a positive effect on skeletal growth and development. Mechanical forces stimulate bone formation and suppress bone resorption, leading to an overall increase in bone mass. Cells from bone and PDL respond to stimulation by activation of mechanosensory signalling systems, cytoskeletal changes, and ECM architecture reorganization to withstand these loads without damage.[93]

In teeth, PDL is involved in the transmission of physical forces resulting from mastication. Under mechanical strain, POSTN mRNA and protein expression were found to be increased in rodent and human PDL fibroblasts, compared to non-stimulated cells. **Wen *et al.* (2010)** investigated whether POSTN protein was expressed in the human PDL *in situ* and the mechanisms regulating POSTN expression in PDL fibroblasts *in vitro.* With immunohistochemistry, POSTN protein was identified in the PDL, with expression lower in teeth with reduced occlusal loading. *In vitro* application of uniaxial cyclic strain to PDL fibroblasts elevated POSTN mRNA levels, depending on the age of the patient. Treatment with TGF-β1 also significantly increased POSTN mRNA levels, an effect attenuated by FAK inhibition. FAK-null fibroblasts contained no detectable POSTN mRNA, even after stimulation with cyclic strain. They concluded that the POSTN protein was strongly expressed in the human PDL. [44]

POSTN knockout mice displayed a unique phenotype demonstrating that POSTN was found to be essential for bone and PDL development and functions in postnatal animals and highlighted its crucial role in mechano-transduction (**Rios *et al.*, 2005, Rios *et al.*, 2008, Bonnet *et al.*, 2009**). [17, 72, 80]

Table 2: POSTN domains involved in interaction with ECM proteins [7]

POSTN domain	Functions	ECM protein
N-terminal EMI domains	Protein interactions and protein multimerization	Collagen I
		Fibronectin
		POSTN
Fas-1 domains with Gla residues	Cell adhesion and protein interactions	BMP-1
		Tenascin
		Calcium
		Integrins
C-terminal end	Potential binding site for glycoproteins	Heparin

In response to mechanical stress, POSTN expression was up-regulated and it activated different cellular pathways to support cell survival, to ensure a correct collagen fibrillogenesis and matrix organization to preserve tissue integrity and function. These data corroborated the results obtained with a rat model of chronic overload showing that isoform 3 was expressed at the beginning of overload primarily in cellular periosteum, articular cartilage, osteoblasts, osteoclasts, and osteocytes in long bone, whereas isoform 2 was induced at a constant high level all along the loading. This rapid increase was concomitant to a transient increase in serum OCN, indicative

of adaptive bone formation, and suggested that POSTN protein expression was associated with anabolic bone changes in response to loading. As the overload continued, immunoexpression of isoform 3 decreased, paralleling with an increase in serum tartrate resistance acid phosphatase, a biological marker of bone resorption, thinning of the growth plate and reduced cortical thickness indicative of a pathological response to excess loading (**Rani *et al.*, 2009**). [39]

POSTN in GCF

Balli *et al*. (2015) investigated POSTN levels in the GCF and serum of patients with periodontal diseases and compared them with those of healthy individuals. Eighty individuals enrolled in the study, were divided into three groups following clinical and radiographic examinations: the healthy group, gingivitis group and CP group. GCF and serum samples were collected and POSTN levels were determined using the ELISA. The total amount and concentration of POSTN decreased in GCF with the progression and severity of the disease from healthy controls to gingivitis and to CP groups and differed significantly. However, there was no significant difference in serum POSTN concentration within all groups. POSTN in GCF negatively correlated with the Gingival Index in the gingivitis groups, whereas it is inversely correlated with the clinical attachment level only in the periodontitis group. When all the clinical groups were examined together, the POSTN concentration inversely correlated with clinical attachment level and Gingival Index. [10]

An observational prospective case–control study by **Padial-Molina M *et al.* (2015)** was designed to characterize POSTN changes over time after periodontal surgery in tissue, GCF and serum by histological, protein and mRNA analyses. The study consisted of groups with healthy and chronic periodontitis, before and after

surgery. Histological analysis showed lower POSTN with a diffuse local distribution pattern in disease patients. Levels of POSTN in GCF increased over time for both groups, more noticeably in the periodontitis subjects. A transient and subtle change in circulating POSTN levels was also noticed. The mRNA POSTN levels contrasted with the protein levels and may indicate the underlying post-transcriptional regulatory process during chronic inflammation. Levels of known periodontal disease biomarkers such as IL-1ß, IL-1α, TNF-α, Macrophage Inflammatory Protein-1α and CRP served as tissue stability markers and complemented the clinical parameters recorded. The transient local increase in GCF POSTN after eliminating the local etiology in periodontally affected sites suggested its importance in the maturation and stability of the connective tissue. The decreasing levels observed as the tissue healed highlight its spatial/temporal significance. [94]

Baeza *et al.*'s (2016) study was to assess the levels and diagnostic accuracy of a set of potential biomarkers of periodontal tissue metabolism in GCF from patients with CP and asymptomatic apical periodontitis. They collected 31 GCF samples from 11 CP patients, 44 GCF samples from 38 asymptomatic apical periodontitis patients and 31 GCF samples from 13 healthy volunteers. MMPs -2 and -9 were determined by zymography; levels of MMP-8 by ELISA and immunofluorometric method and myeloperoxidase (MPO) by ELISA. IL-1, IL-6, TNF-α, Dickkopf-1, Osteonectin, POSTN, tartrate resistant acid phosphatase (TRAP)-5 and OPG were determined by a multiplex quantitative panel. The MMP-9 and MMP-8 were higher in CP, followed by asymptomatic apical periodontitis, versus healthy individuals. ProMMP-2, MPO, IL-1, IL-6, POSTN, TRAP-5 and OPG were

significantly higher in CP when compared with asymptomatic apical periodontitis and healthy patients. [95]

In their study, **Aral *et al.* (2016)** aimed to evaluate the levels of POSTN in CP and AgP compared to non-periodontitis. Individuals were submitted to GCF and saliva sampling. Periodontal examination consisted of Plaque Index, Gingival Index, PD, bleeding on probing, and CAL measurements. Assays for POSTN were performed by ELISA. Periodontitis patients presented more severe clinical indices compared to the healthy group. The mean GCF level of POSTN was lowest in the AgP group as compared to the other groups and was lower in the CP group as compared to the healthy group. Increased levels of POSTN were observed in the saliva of patients with AgP as compared to the CP and healthy groups. There was a negative relationship between GCF POSTN levels and clinical parameters, whereas a positive correlation was observed between salivary POSTN levels and full-mouth Gingival Index and CAL scores. Saliva had been used to follow changes concerning the whole mouth, and gingival inflammation and disease severity might be the cause of increased salivary POSTN in the AgP group. Together with local production of POSTN in periodontal tissues, high salivary POSTN levels in AgP group might be caused by serum POSTN found in saliva by spontaneous bleeding. [11]

Biostimulation utilizing low-level laser therapy influences periodontal ligament fibroblast proliferation. **Kumaresan *et al*. (2016)** 's study was conducted with the objective of estimating POSTN levels in CP patients following low-level laser therapy as an adjunct to root surface debridement. The study included 30 periodontally healthy participants and 60 CP participants. Based on the therapeutic intervention, CP

patients were allocated to either root surface debridement or to root surface debridement with low-level laser therapy group. Clinical parameters and GCF POSTN levels were assessed at the baseline and at the 3rd month. POSTN levels were significantly lower in CP patients when compared to healthy individuals at the baseline. Following nonsurgical periodontal treatment, POSTN levels significantly increased in both CP groups, when compared to baseline values. Comparison of mean POSTN levels between both the treatment groups showed a significant increase in low-level laser therapy group than root surface debridement at the 3rd month. [96]

Akman *et al.* (2017) detected the levels of POSTN in peri-implant sulcular fluid and GCF and evaluated the relationship between POSTN, Pyridinoline cross-linked carboxyterminal telopeptide of type 1 collagen and C-terminal crosslinked telopeptide of type 1 collagen levels and clinical inflammatory symptoms and duration of functional loading. The study population was constituted of 9 women and 4 men. 20 'bone-level designed' dental implants placed in the molar or premolar sites, without any signs of peri-implant bone loss and with a restoration in function for at least 12 months and 20 contralateral natural teeth were included in the study as controls. Clinical parameters and restoration dates of the implants were recorded. The peri-implant sulcular fluid and GCF Pyridinoline cross-linked carboxyterminal telopeptide of type 1 collagen, C-terminal crosslinked telopeptide of type 1 collagen and POSTN levels were evaluated using ELISA. The Pyridinoline cross-linked carboxyterminal telopeptide of type 1 collagen, C-terminal crosslinked telopeptide of type 1 collagen and POSTN levels were similar between implant and natural teeth groups. There were no statistically significant differences between peri-implant sulcular fluid and GCF values.

When implants were grouped as healthy (Gingival Index = 0) and inflamed (Gingival Index ≥ 0), Pyridinoline cross-linked carboxyterminal telopeptide of type 1 collagen levels and peri-implant sulcular fluid volume were lower in healthy implants compared to the inflamed group. Both, POSTN and C-terminal crosslinked telopeptide of type 1 collagen levels were negatively correlated with functioning time suggesting less bone remodeling around dental implants at later stages of functioning. [97]

MATERIALS AND METHODS

STUDY DESIGN:

39 subjects (13 healthy, 13 CP, 13 AgP) were recruited from the Department of Periodontics, Sri Ramakrishna Dental College and Hospital, Coimbatore. The study protocol was approved by the ethical committee, Sri Ramakrishna Dental College and Hospital, Coimbatore.

CRITERIA OF INCLUSION:

SELECTION OF CONTROLS

GROUP - I

- Healthy controls - Subjects with periodontal probing depth < 3 mm, no bleeding on probing and no radiographic evidence of bone loss.

SELECTION OF CASES

GROUP-II

- Generalized (or) localised mild, moderate or severe chronic periodontitis patients (According to Armitage criteria)

GROUP - III

- Localised (or) generalized aggressive periodontitis patients – (According to Lang's criteria)

CRITERIA OF EXCLUSION:

- Pregnancy and Lactation
- Current smoker or former smoker
- Periodontal therapies during the previous 6 months

- Any drug intake during the previous 3 months
- Any systemic condition that could compromise periostin levels such as Diabetes mellitus, cancer, ventricular hypertrophy.

ARMAMENTARIUM:

SAMPLE COLLECTION AND STORAGE:

- Dental mouth mirror
- William's periodontal probe
- Explorer
- Tweezer
- Cotton rolls
- Universal curettes 2R/2L, 4R/4L
- Head cap
- Surgical mask
- Gloves
- Microcapillary pipettes (10µl, Drummond Scientific Company, Delaware County, Pennsylvania, United States)
- 2ml Polypropylene tubes
- Aluminium foil
- Ultra-low temperature freezer (-80ºC)

SAMPLE PROCESSING:

- ELISA kit for detection of POSTN

CLINICAL EXAMINATION:

During examination of the patient, the following clinical parameters were assessed:

Plaque Index:

The full mouth plaque was assessed using the criteria of the Plaque Index system given by **Silness and Loe (1967).** [12]

Modified Sulcular Bleeding Index (mSBI):

The sulcular bleeding was assessed using the criteria given by **Mombelli *et al*. (1987)**. [13]

Conventional probing depth:

The probing depth was assessed on each tooth from the gingival margin to base of the sulcus/pocket using William's periodontal probe at 6 specific surfaces per tooth (distobuccal, midbuccal, mesiobuccal, distolingual, midlingual and mesiolingual surfaces). [14]

Clinical attachment level (CAL):

The clinical attachment level was recorded from cemento-enamel junction to the base of the gingival sulcus/periodontal pocket using William's periodontal probe at all the six sites as mentioned for probing depth. [15]

GCF COLLECTION:

GCF samples were obtained using microcapillary pipettes. GCF from sites with the greatest CAL and demonstrating maximum bleeding score as assessed by the mSBI in subjects with chronic and aggressive periodontitis; and from the most convenient site in healthy individuals. The site was isolated by means of cotton/gauze.

The plaque along with the supragingival calculus was removed with a universal curette to avoid contamination and blocking of the microcapillary pipette by plaque. The sulcular areas were gently air dried. A colour coded, calibrated (10µl) volumetric microcapillary pipette (Drummond, Broomall, USA) ™ was placed at the entrance of the gingival crevice. The GCF samples which were contaminated with blood, saliva or exudate were discarded and fresh samples were collected. Each microcapillary pipette containing 2µl GCF was wrapped in aluminium foil, placed inside separate sterile tubes and stored at -80°C in an ultra-low temperature freezer (Thermo Fisher Scientific India Pvt.td, Mumbai, India), until further analysis of POSTN by ELISA procedure.

ELISA Analysis for GCF Samples:

The contents of the kit and the samples were brought to room temperature. For duplication, the samples were divided into two (1µl each) and diluted to 2µl. A 50µl standard was added to the standard well. A 40µl of diluted sample was added to sample wells and then 10µl anti-POSTN antibody was added to sample wells, then 50µl streptavidin-HRP was added to sample wells and standard wells but not added in the blank control well and thoroughly mixed. The plate was covered with a sealer and incubated for 60 minutes at 37°C. The sealer was removed and the plate was washed 5 times with wash buffer. The wells were soaked with at least 0.35 ml wash buffer for 30 seconds to 1 minute for each wash. The plate was blotted onto paper towels or other absorbent material. A 50µl substrate solution A was added to each well and then 50µl substrate solution B was added to each well. The plate covered with a new sealer was incubated for 10 minutes at 37°C in the dark. A 50µl stop solution was added to each well, the blue color will change into yellow immediately. The optical density

value of each well was determined immediately using a microplate reader set to 450 nm within 30 min after adding the stop solution. The concentrations of POSTN were expressed as picograms per microlitre (pg/μl).

SRI RAMAKRISHNA DENTAL COLLEGE AND HOSPITAL,

COIMBATORE.

DEPARTMENT OF PERIODONTICS

ASSESSMENT OF PERIOSTIN LEVELS IN GINGIVAL CREVICULAR FLUID OF PATIENTS WITH CHRONIC PERIODONTITIS AND AGGRESSIVE PERIODONTITIS AND COMPARISON WITH THE HEALTHY SUBJECTS

FORM I - SCREENING PROFORMA

NAME: O.P. No.: DATE:

AGE: GENDER:

POSTAL ADDRESS:

MOBILE NUMBER:

OCCUPATION:

CRITERIA OF INCLUSION:

SELECTION OF CONTROLS

GROUP - I

- Healthy controls - Subjects with periodontal probing depth < 3 mm, no bleeding on probing and no radiographic evidence of bone loss.

SELECTION OF CASES

GROUP-II

- Generalized (or) localised mild, moderate or severe chronic periodontitis patients (According to Armitage criteria)

GROUP - III

- Localised (or) generalized aggressive periodontitis patients – (According to Lang's criteria)

CRITERIA OF EXCLUSION:

- Pregnancy and Lactation
- Current smoker or former smoker
- Periodontal therapies during the previous 6 months
- Any drug intake during the previous 3 months
- Any systemic condition that could compromise periostin levels such as diabetes mellitus, cancer, ventricular hypertrophy.

CASE/CONTROL: CASE ☐ CONTROL ☐

WRITTEN INFORMED CONSENT OBTAINED: YES ☐ NO ☐

FORM II- HISTORY PROFORMA

CHIEF COMPLAINT WITH DURATION:

	Absent	Present	Don't Know
1. Bleeding gums	☐	☐	☐
2. Pain in gums	☐	☐	☐
3. Swollen gums	☐	☐	☐
4. Pus discharge from gums	☐	☐	☐
5. Mobility	☐	☐	☐
6. Dentinal Hypersensitivity	☐	☐	☐

7. Any other complaints (Specify):

HISTORY OF PRESENT ILLNESS:

PAST DENTAL HISTORY:

MEDICAL HISTORY:

FAMILY HISTORY:

PERSONAL HISTORY:

1. Diet: Veg ☐ Mixed ☐

2. Brushing habit:

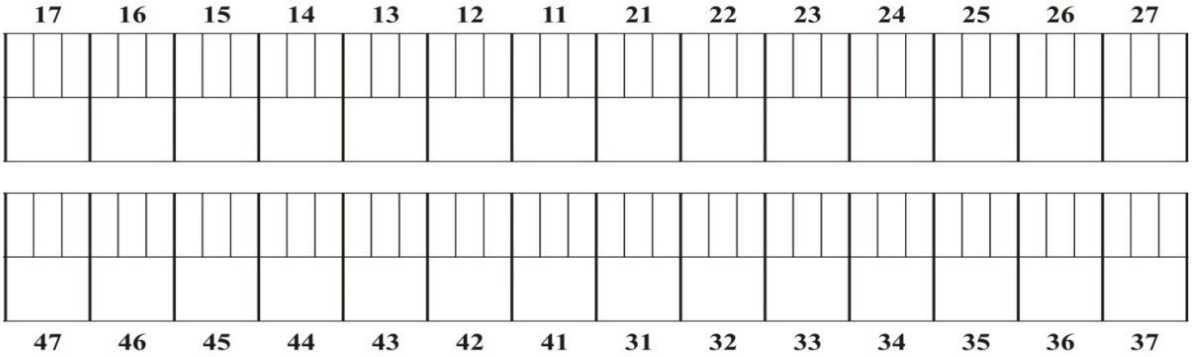
3. Smoking: Yes ☐ No ☐

4. Any other (specify):

FORM III- CLINICAL PARAMETER ASSESSMENT

A) INDICES

a) PLAQUE INDEX (Silness and Loe, 1967)



Calculation: $\frac{\text{Sum of score of each teeth}}{\text{Total number of teeth examined}}$

Inference:

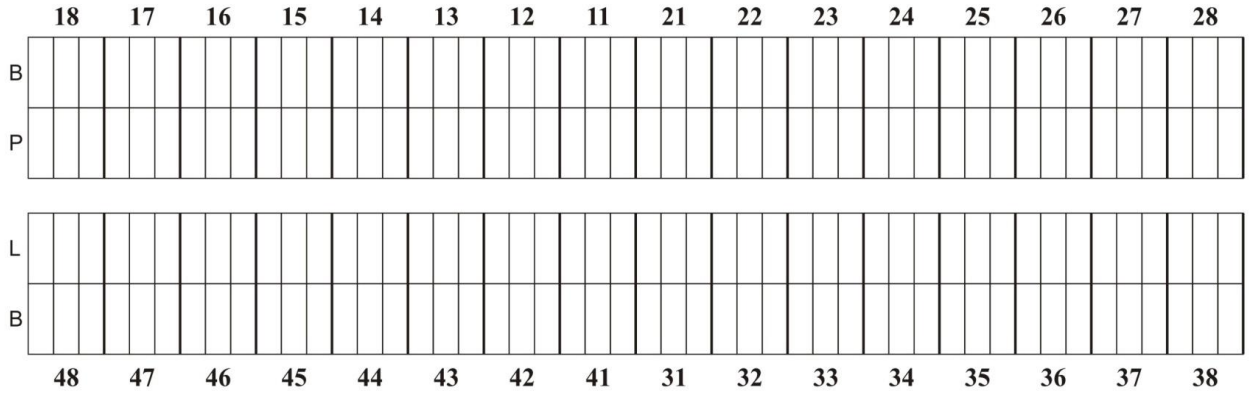
b) MODIFIED SULCULAR BLEEDING INDEX (Mombelli et. al, 1987)

18	17	16	15	14	13	12	11	21	22	23	24	25	26	27	28

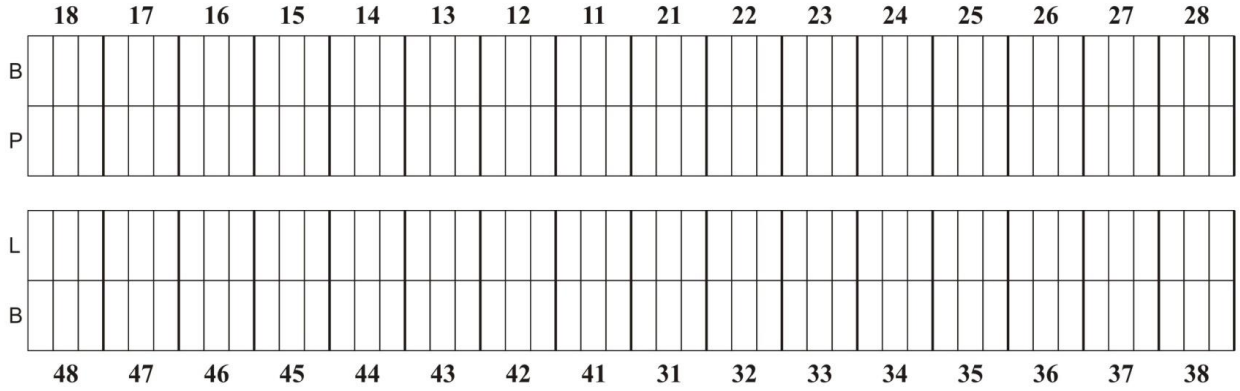
48	47	46	45	44	43	42	41	31	32	33	34	35	36	37	38

B) PERIODONTAL STATUS

a) PROBING DEPTH (CONVENTIONAL PROBING METHOD)



b) CLINICAL ATTACHMENT LEVEL:



GCF collection site:

FORM IV LABORATORY INVESTIGATION

Collection of GCF samples for analysis of POSTN by ELISA procedure.

FORM V - CONSENT FORM

CERTIFICATE BY INVESTIGATOR

The samples are being collected solely for the purpose of research. No harm shall come to the patient by providing the samples. I certify that I have disclosed all details about the study in the terms easily understood by the patient.

Dated: _____ **Signature:**

Name:

CONSENT BY SUBJECT

I have been informed to my satisfaction, by the attending dental surgeon, the purpose of the clinical examination and the laboratory investigation.

The details of the above study have been explained in the language understood by me.

Dated: _____ **Signature or thumb impression**

ஒப்புதல் படிவம்

நான் புரிந்து கொள்ளக்கூடிய மொழியில் என்னை பரிசோதிக்கும் பல் மருத்துவரால், இந்த மருத்துவ பரிசோதனை மற்றும் ஆய்வக ஆய்வுக்கான காரணத்தை தெரிவிக்கப்பட்டுள்ளேன். இந்த ஆய்வில் பங்கேற்க, நான் முழு மனதுடன் சம்மத்திக்கிறேன்.

தேதி : _____ **கையெழுத்து அல்லது கை எண்ணம்**

FIGURES

Fig. 4: Armamentarium

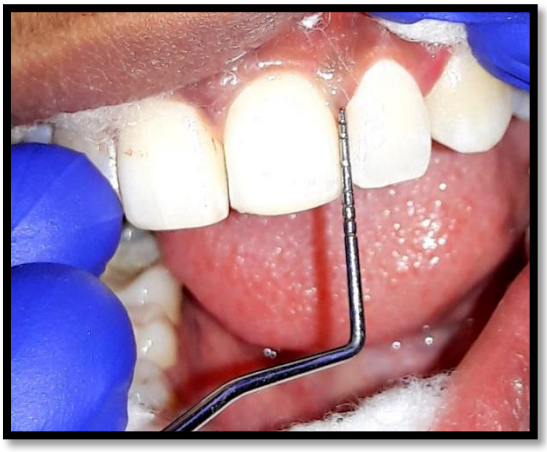


Fig. 5: Clinical image of a healthy subject

Fig. 6: Radiograph of the sampled site

Fig. 7: GCF collection from a healthy subject

Fig. 8: Clinical image of a patient with CP

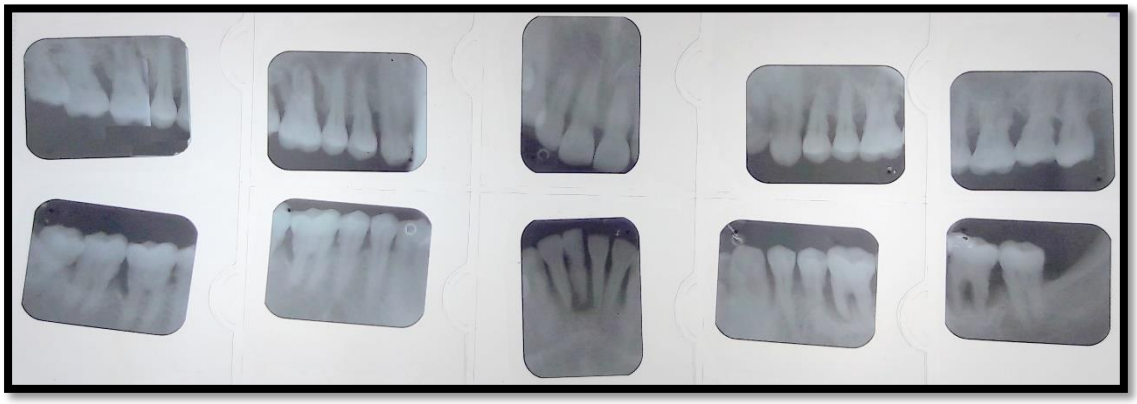


Fig. 9: Radiograph of a patient with CP

Fig. 10: GCF collection from a patient with CP

Fig. 11: Clinical image of a patient with AgP

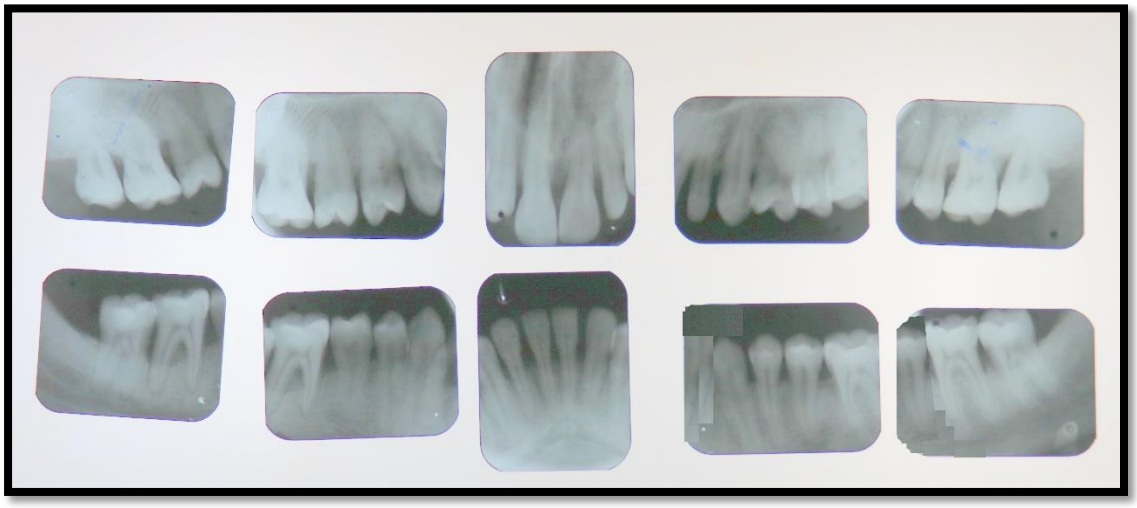


Fig. 12: Radiograph of a patient with AgP

Fig. 13: GCF collection from a patient with AgP

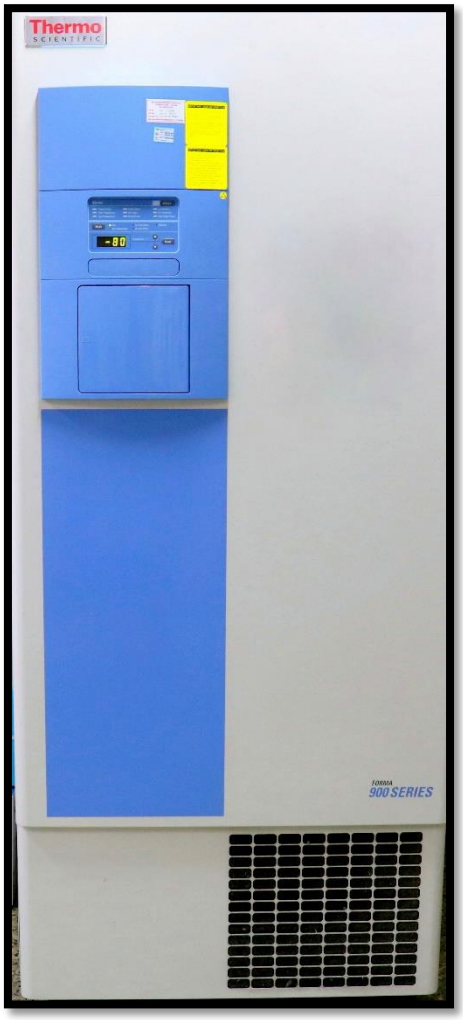


Fig. 14: Ultra-low temperature freezer for storing collected samples

Fig. 15: ELISA kit

Fig. 16: Dispension of samples into wells

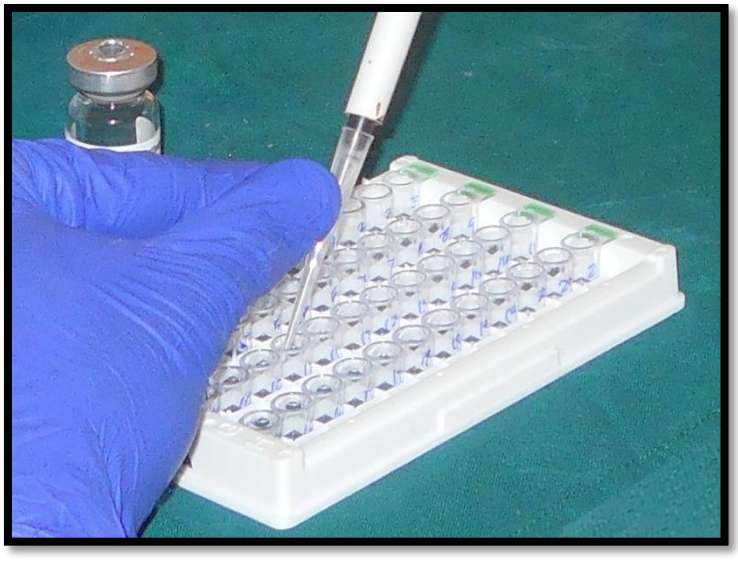


Fig. 17: Addition of antibody solution into wells

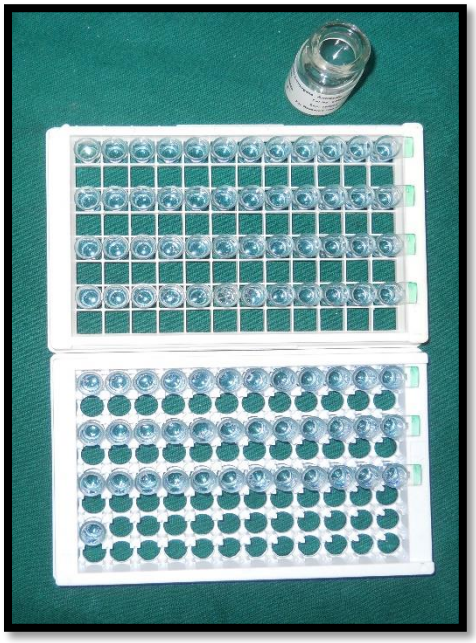


Fig. 18: After addition of antibody solution into wells

Fig. 19: For Incubation

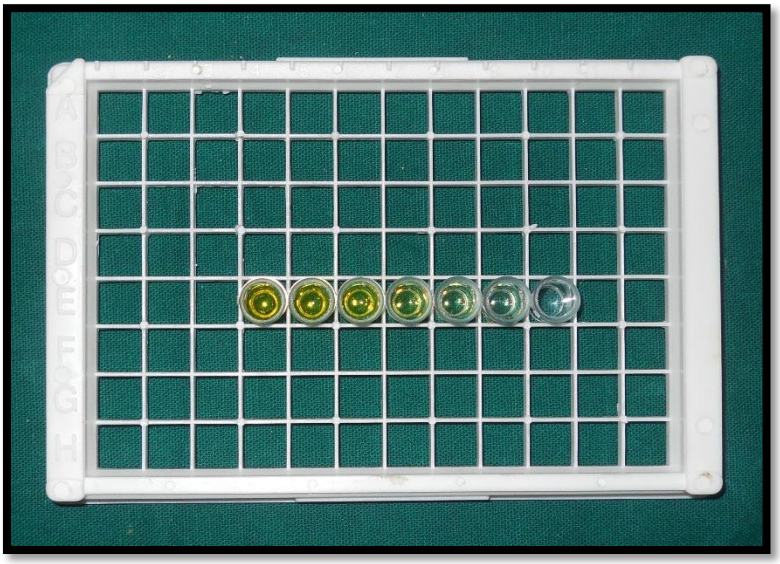


Fig. 20: Colour change in standard wells

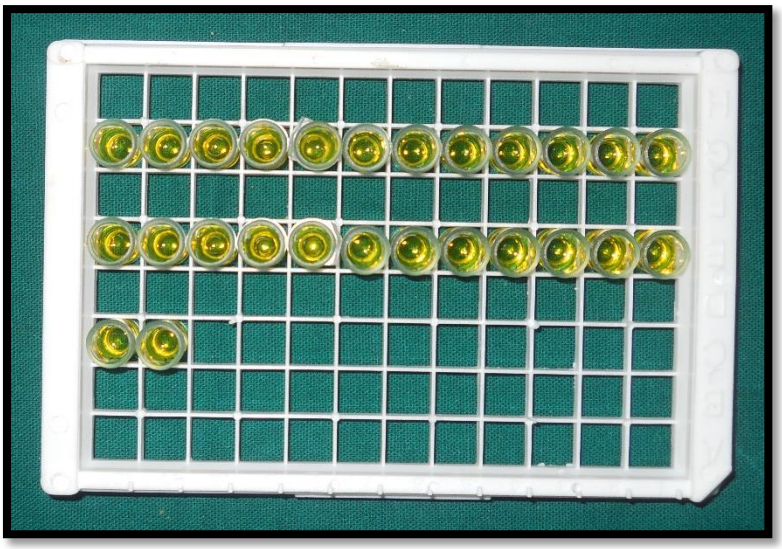


Fig. 21: Colour change in wells with GCF samples of healthy subjects

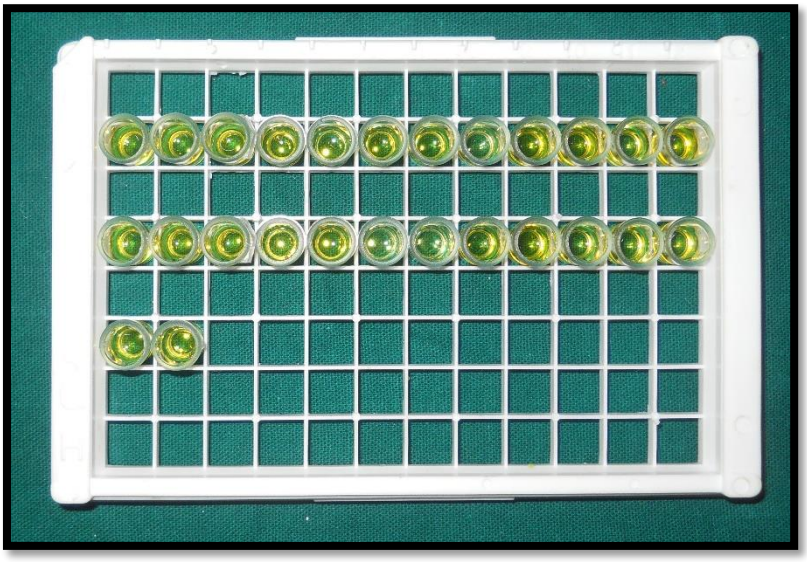


Fig. 22: Colour change in wells with GCF samples of CP patients

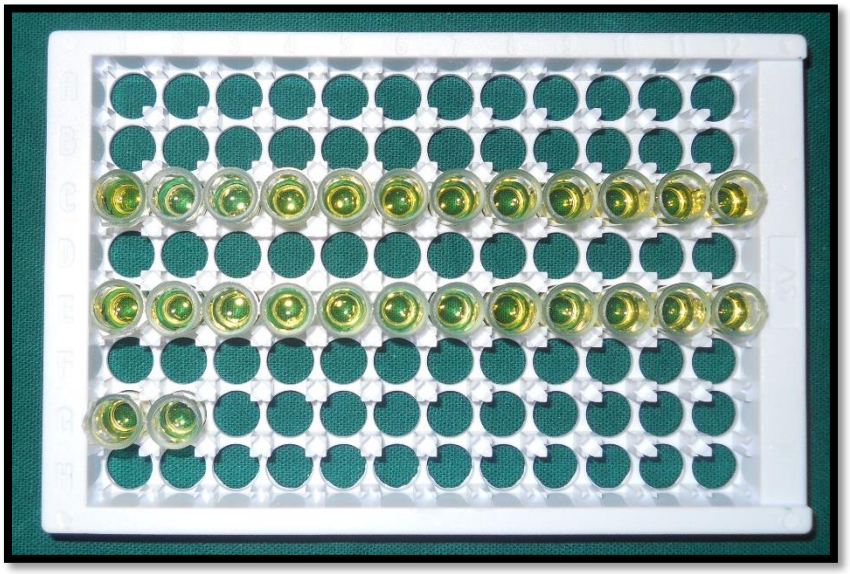


Fig. 23: Colour change in wells with GCF samples of AgP patients

Fig. 24: Reading the plate using ELISA reader

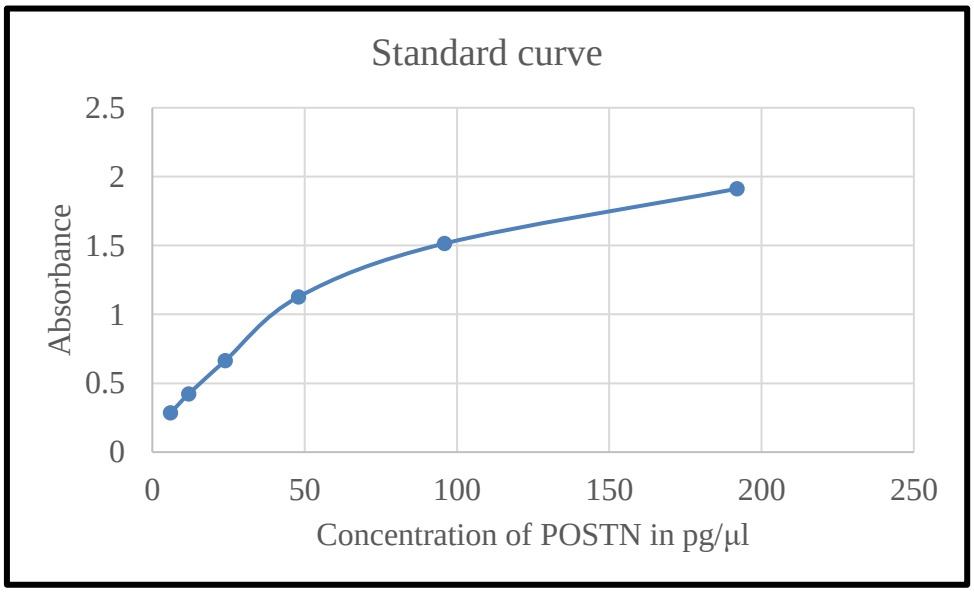


Fig. 25: Standard curve for human POSTN

RESULTS

Statistical analysis:

The sample size was calculated using Sampling software - G.Power Version 3.1.9.2 with alpha error of 5% and power of 95% and it was found to be 12 in each group. One extra sample was collected in each group as a standby for avoiding any errors while processing and hence the total number of samples was 13 in each group. Statistical analysis was performed using a commercially available software (SPSS 16.0; SPSS Inc., Chicago, IL, USA). The Shapiro–Wilk test was used to investigate whether the data were normally distributed. Variables in the three groups were compared using one-way analysis of variance (ANOVA) and for multiple comparisons, Tukey HSD was used. Comparisons of the level of POSTN in GCF, age, Plaque Index, mSBI and CAL were analysed and correlated using Pearson correlation in healthy, CP, AgP groups.

Results:

All GCF samples in three groups showed the presence of POSTN. The values of levels of POSTN in GCF were expressed in picogram/microliter (pg/µl). The demographics, clinical parameters and GCF POSTN levels are summarized in Table 3, 4 and 5 respectively and ANOVA comparison in Table 6.

The mean age in healthy, CP and AgP groups was 24.69, 43.46 and 26.85 years respectively (Table 3) (Graph 1) with a statistically significant difference between all the groups (Table 6 - F value: 41.36; $p < 0.05$). When the mean age was compared among groups, there was a statistically significant difference among healthy and CP groups (Table 7 – mean difference: 18.77 and $p < 0.05$) (Graph 4), among CP and AgP groups (Table 7 - mean difference: 16.62 and $p < 0.05$) (Graph 4).

There was no statistically significant difference when age was compared among healthy and AgP groups (Table 7 – mean difference: 2.15 and $p = 0.610$) (Graph 4).

Among the 13 individuals in each group, 6 males and 7 females were included in healthy group, 4 males and 9 females in CP group and 8 males and 5 females in AgP group. In total, 18 males and 21 females were included in the study (Table 3).

The mean Plaque Index score was 0.24, 1.18 and 0.96 in healthy, CP and AgP groups respectively (Table 4) (Graph 2A). There was a statistically significant difference between the groups (Table 6 - F value: 28.17; $p < 0.05$). When the mean Plaque Index was compared among groups, there was a statistically significant difference among healthy and CP groups (Table 8 - mean difference: 0.93 and $p < 0.05$) (Graph 5), among healthy and AgP groups (Table 8 - mean difference: 0.72 and $p < 0.05$) (Graph 5) and no statistically significant difference among CP and AgP groups (Table 8 - mean difference: 0.22 and $p = 0.232$) (Graph 5).

The mean values of mSBI percentage for the healthy, CP, AgP groups were 0%, 65.7% and 69.7% respectively (Table 4) (Graph 2B). There was a statistically significant difference between the groups (Table 6 - F value: 66.04; $p < 0.05$). When the mSBI was compared among groups, there was a statistically significant difference among healthy and CP groups (Table 9 - mean difference: 65.70 and $p < 0.05$) (Graph 6), among healthy and AgP groups (Table 9 - mean difference: 69.71 and $p < 0.05$) (Graph 6) and no statistically significant difference among CP and AgP groups (Table 9 - mean difference: 4.01 and $p = 0.827$) (Graph 6).

The mean values of CAL for the healthy, CP, AgP groups were 0, 4.5 mm and 5.17 mm respectively (Table 4) (Graph 2C). There was a statistically significant difference between the groups (Table 6 - F value: 34.64; $p < 0.05$). When

the mean CAL was compared among groups, there was a statistically significant difference among healthy and CP groups (Table 10 - mean difference: 4.5 and $p < 0.05$) (Graph 7), among healthy and AgP groups (Table 10 - mean difference: 5.17 and $p < 0.05$) (Graph 7) and no statistically significant difference among CP and AgP groups (Table 10 - mean difference: 0.68 and $p = 0.582$) (Graph 7).

The mean levels of total POSTN in GCF were 182.41 pg/µl, 79.87 pg/µl and 49.28 pg/µl for the healthy, CP, AgP groups respectively (Table 5, Graph 3). There was a statistically significant difference between the groups (Table 6 - F value: 89.45; $p < 0.05$). The mean levels of total POSTN in GCF were significantly lower in the CP and AgP groups than in the healthy controls. The amount of POSTN in GCF decreased by 56% in CP group and by 73% in AgP group when compared to healthy group. There was a statistically significant difference among healthy and CP groups (Table 11 - mean difference: 102.54 and p: $p < 0.05$) (Graph 8), among healthy and AgP groups (Table 11 - mean difference: 133.13 and $p < 0.05$) (Graph 8) and also among CP and AgP groups (Table 11 - mean difference: 30.59 and $p < 0.05$) (Graph 8).

The Pearson correlation is shown in Table 12, Graph 9. When all clinical groups were examined together, there were negative correlations between POSTN levels in GCF and age, mSBI, Plaque Index, CAL, that is, the POSTN levels in GCF were inversely related to age, mSBI, Plaque Index, CAL. It was found that the negative correlation between the POSTN levels in GCF and age ($r = -0.303$; $p = 0.061$) was not statistically significant and the negative correlations between the POSTN levels in GCF and mSBI, Plaque Index, CAL ($r = -0.788$, $r = -0.655$, $r = -0.691$ respectively; $p < 0.01$) were statistically significant (2 - tailed). Also, in healthy group, negative correlations were found between the POSTN levels in GCF and age ($r = -0.302$; $p > 0.317$), and the

POSTN levels in GCF and Plaque Index ($r = -0.146$; $p > 0.635$), but the correlations were not statistically significant. The correlations between the POSTN levels in GCF and mSBI and the POSTN levels in GCF and CAL were not found. In CP group, negative correlations were found between the POSTN levels in GCF and mSBI ($r = -0.204$; $p = 0.504$), and the POSTN levels in GCF and Plaque Index ($r = -0.373$; $p = 0.209$), but the correlations were not statistically significant. The correlations between the POSTN levels in GCF and age ($r = 0.333$; $p = 0.267$) and the POSTN levels in GCF and CAL ($r = 0.370$; $p = 0.214$) were positive and not statistically significant. In AgP group, a negative correlation was found between the POSTN levels in GCF and age ($r = -0.280$; $p = 0.354$) which was not statistically significant. But, the correlations between the POSTN levels in GCF and mSBI ($r = 0.499$; $p = 0.082$), the POSTN levels in GCF and Plaque Index ($r = 0.228$; $p = 0.454$), and the POSTN levels in GCF and CAL ($r = 0.427$; $p = 0.145$) were found to be positive and not statistically significant.

Table 3: Demographic parameters of study population

	Group	N	Mean	Standard Deviation
Age in years	Healthy	13	24.6923	3.72793
	Chronic Periodontitis	13	43.4615	7.01920
	Aggressive Periodontitis	13	26.8462	6.02559
	Total	39	31.6667	10.17824
Gender		Male	Female	Total
	Healthy	6	7	13
	Chronic Periodontitis	4	9	13
	Aggressive Periodontitis	8	5	13
	Total	18	21	39

Table 4: Clinical parameters measured in the study population

	Group	N	Mean	Standard Deviation
Plaque Index	Healthy	13	0.2431	0.11434
	Chronic Periodontitis	13	1.1769	0.23157
	Aggressive Periodontitis	13	0.9600	0.51374
	Total	39	0.7933	0.51753
mSBI in percentage	Healthy	13	0	0
	Chronic Periodontitis	13	65.6985	17.97498
	Aggressive Periodontitis	13	69.7100	24.11623
	Total	39	45.1362	36.52242
CAL in mm	Healthy	13	0	0
	Chronic Periodontitis	13	4.4992	0.97271
	Aggressive Periodontitis	13	5.1746	2.82161
	Total	39	3.2246	2.86825

mSBI – modified Sulcular Bleeding Index score; CAL – Clinical Attachment Level.

Table 5: The POSTN levels in GCF

	Group	N	Mean	Standard Deviation
POSTN value in GCF (pg/µl)	Healthy	13	182.4115	35.50758
	Chronic Periodontitis	13	79.8692	25.91235
	Aggressive Periodontitis	13	49.2808	13.70401
	Total	39	103.85	63.21700

GCF – Gingival Crevicular fluid; POSTN – Periostin.

Table 6: ANOVA – Comparison of age, Plaque Index, mSBI, CAL, GCF POSTN values between the groups

Parameters	ANOVA	F	p
Age in years	Between Groups	41.362	< 0.05*
Plaque Index	Between Groups	28.174	< 0.05*
mSBI in percentage	Between Groups	66.041	< 0.05*
CAL in mm	Between Groups	34.644	< 0.05*
POSTN value in GCF (pg/µl)	Between Groups	89.448	< 0.05*

*. All parameters were statistically significant between the groups

Table 7: Tukey HSD – Comparison of age among the groups

Dependent Variable	Groups	Groups	Mean Difference	Std. Error	p
Age in years	Healthy	Chronic Periodontitis	18.76923*	2.25859	< 0.05*
	Chronic Periodontitis	Aggressive Periodontitis	16.61538*	2.25859	< 0.05*
	Healthy	Aggressive Periodontitis	2.15385	2.25859	0.610

*. The mean difference is significant at the 0.05 level.

Table 8: Tukey HSD – Comparison of Plaque Index among the groups

Dependent Variable	Groups	Groups	Mean Difference	Std. Error	p
Plaque Index	Healthy	Chronic Periodontitis	0.93385*	0.13021	< 0.05*
	Healthy	Aggressive Periodontitis	0.71692*	0.13021	< 0.05*
	Chronic Periodontitis	Aggressive Periodontitis	0.21692	0.13021	0.232

*. The mean difference is significant at the 0.05 level.

Table 9: Tukey HSD – Comparison of mSBI among the groups

Dependent Variable	Groups	Groups	Mean Difference	Std. Error	p
mSBI in percentage	Healthy	Chronic Periodontitis	65.69846*	6.81135	< 0.05*
	Healthy	Aggressive Periodontitis	69.71000*	6.81135	< 0.05*
	Chronic Periodontitis	Aggressive Periodontitis	4.01154	6.81135	0.827

*. The mean difference is significant at the 0.05 level.

Table 10: Tukey HSD – Comparison of CAL among the groups

Dependent Variable	Groups	Groups	Mean Difference	Std. Error	p
CAL in mm	Healthy	Chronic Periodontitis	4.49923*	0.67587	< 0.05*
	Healthy	Aggressive Periodontitis	5.17462*	0.67587	< 0.05*
	Chronic Periodontitis	Aggressive Periodontitis	0.67538	0.67587	0.582

*. The mean difference is significant at the 0.05 level.

Table 11: Tukey HSD – Comparison of GCF POSTN values among the groups

Dependent Variable	Groups	Groups	Mean Difference	Std. Error	p
POSTN value in GCF(pg/µl)	Healthy	Chronic Periodontitis	102.54231*	10.42688	< 0.05*
	Healthy	Aggressive Periodontitis	133.13077*	10.42688	< 0.05*
	Chronic Periodontitis	Aggressive Periodontitis	30.58846*	10.42688	< 0.05*

*. The mean difference is significant at the 0.05 level.

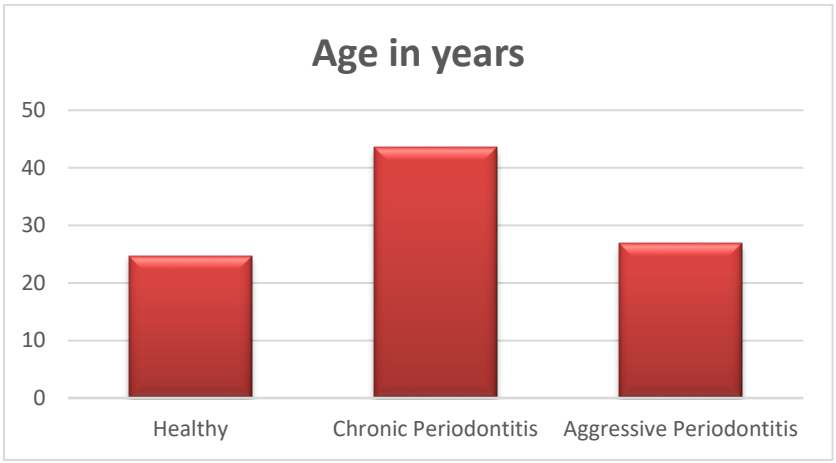
Table 12: Pearson correlation between the levels of POSTN in GCF and age, mSBI, Plaque Index and CAL

POSTN value in GCF		Age	mSBI	Plaque Index	CAL
All groups N = 39	Pearson Correlation (r)	-0.303	-0.788**	-0.655**	-0.691**
	Significance (p) (2-tailed)	0.061	< 0.01**	< 0.01**	< 0.01**
Healthy N = 13	Pearson Correlation (r)	-0.302	- [a]	-0.146	- [a]
	Significance (p) (2-tailed)	0.317	-	0.635	-
CP N = 13	Pearson Correlation (r)	0.333	-0.204	-0.373	0.370
	Significance (p) (2-tailed)	0.267	0.504	0.209	0.214
AgP N = 13	Pearson Correlation (r)	-0.280	0.499	0.228	0.427
	Significance (p) (2-tailed)	0.354	0.082	0.454	0.145

a. Cannot be computed because at least one of the variables is constant

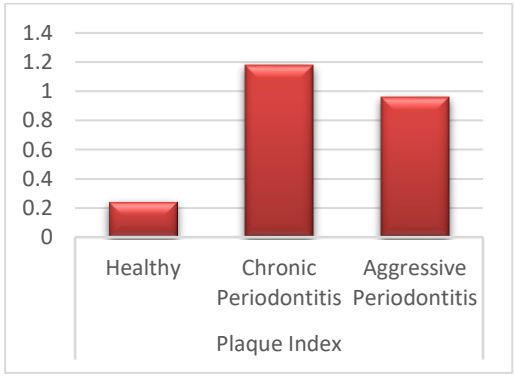
**. Correlation is significant at the 0.01 level (2-tailed).

Graph 1 – Age

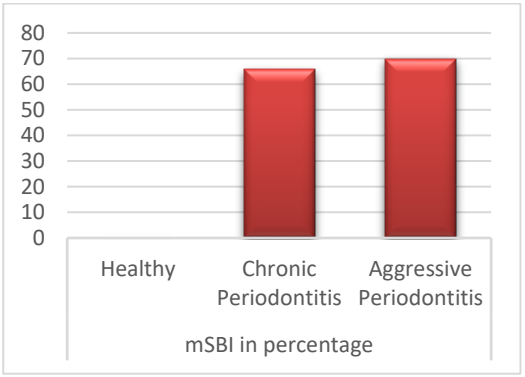


Graph 2 – Clinical parameters (2A - Plaque Index, 2B - mSBI, 2C - CAL)

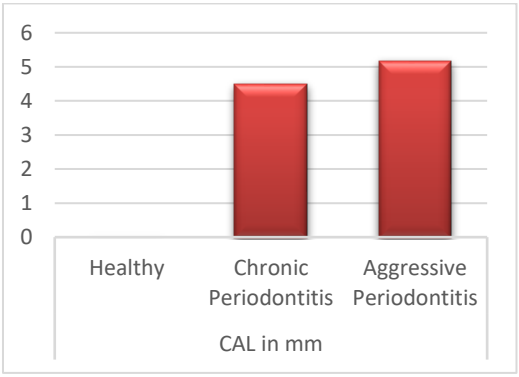
2A



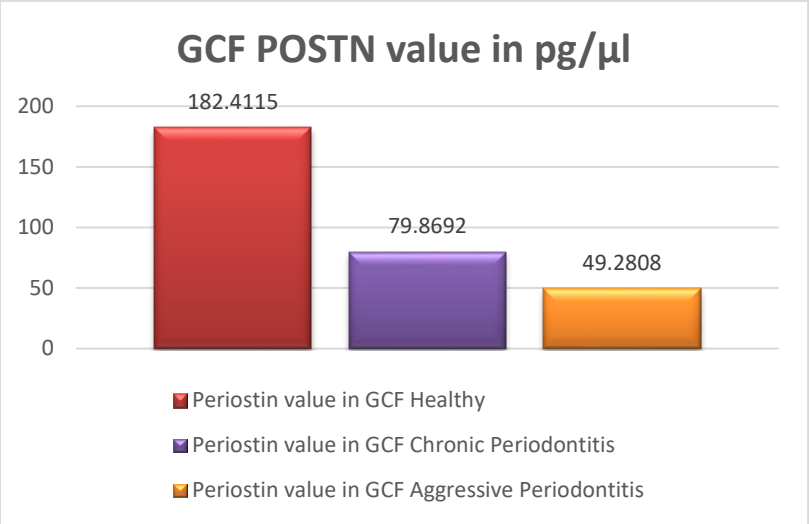
2B



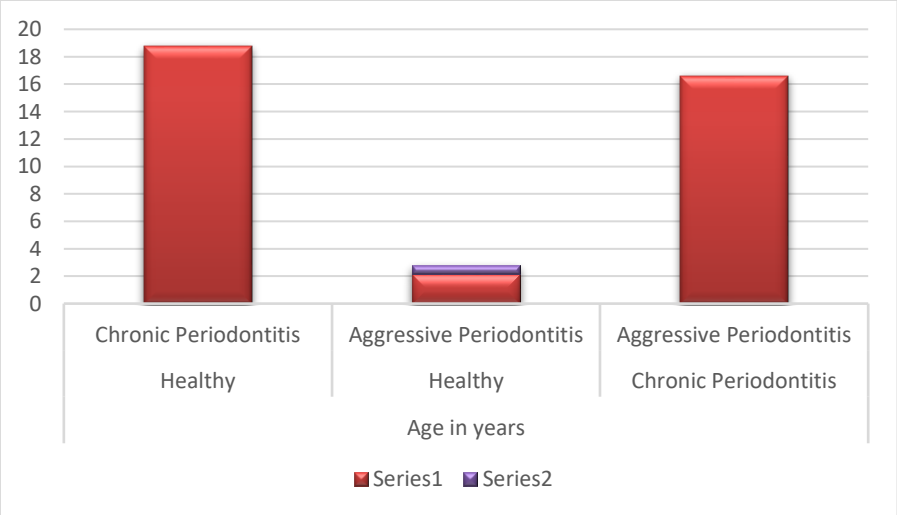
2C



Graph 3 – GCF POSTN value



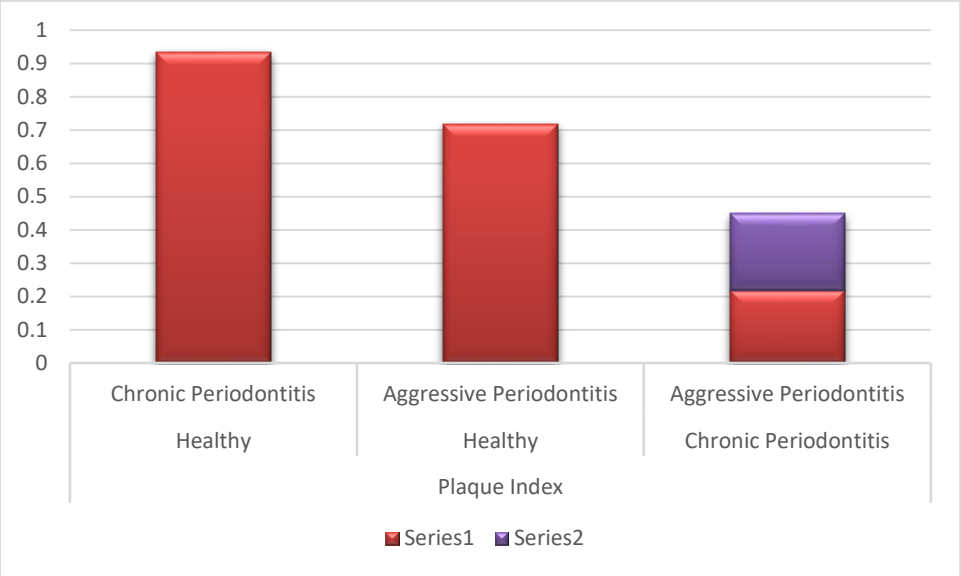
Graph 4 - Tukey HSD - Comparison of age among the groups



Series1: Mean Difference; Series2: p value

*. The mean difference is significant at the 0.05 level.

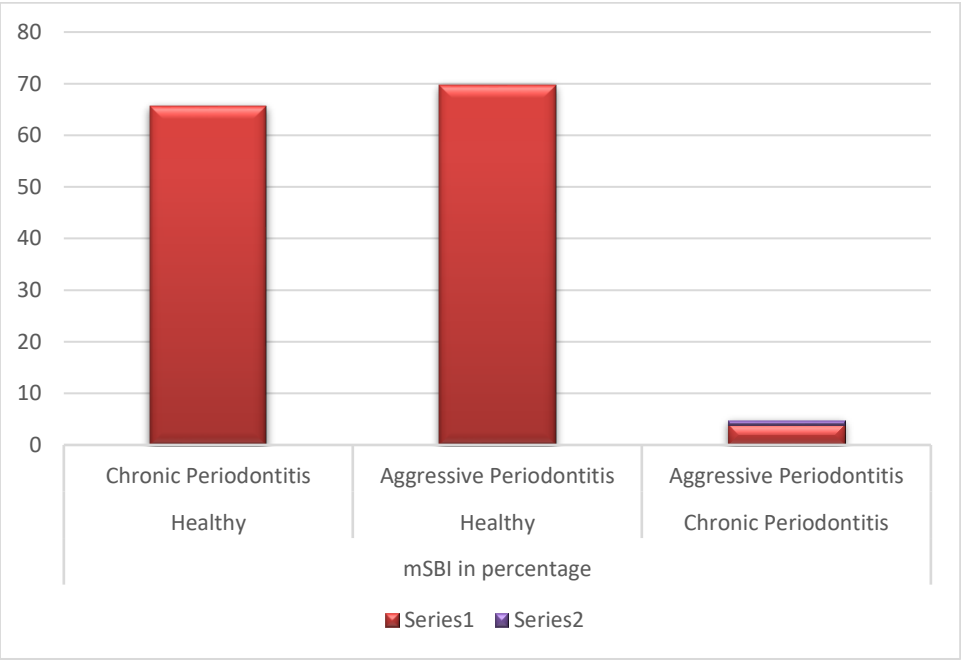
Graph 5 - Tukey HSD - Comparison of Plaque Index among the groups



Series1: Mean Difference; Series2: p value

*. The mean difference is significant at the 0.05 level.

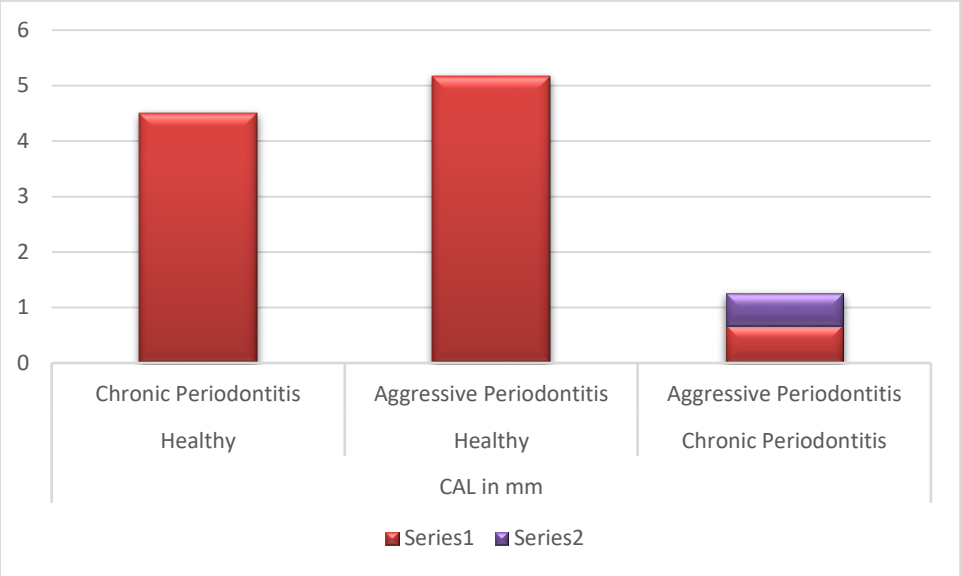
Graph 6 - Tukey HSD - Comparison of mSBI among the groups



Series1: Mean Difference; Series2: p value

*. The mean difference is significant at the 0.05 level.

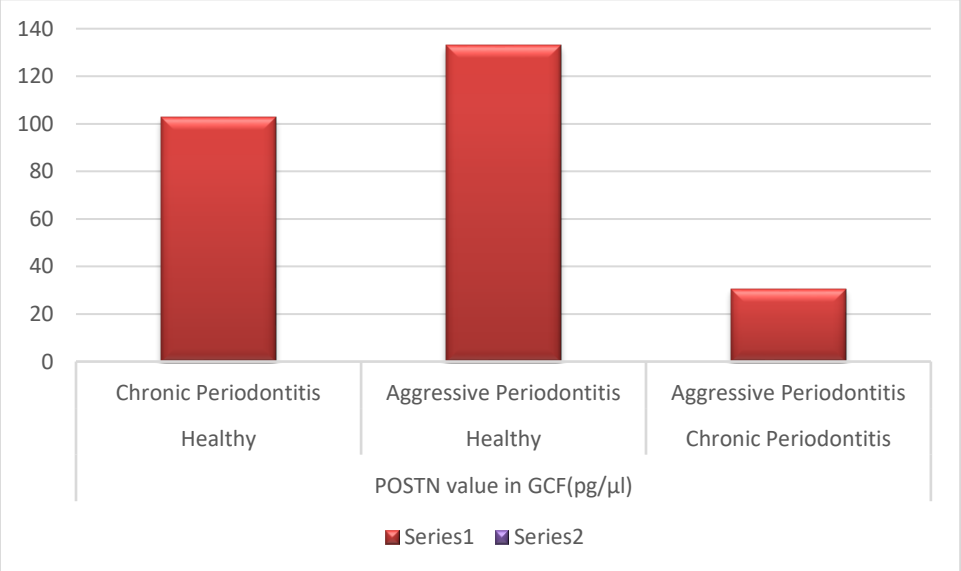
Graph 7 - Tukey HSD - Comparison of CAL among the groups



Series1: Mean Difference; Series2: p value

*. The mean difference is significant at the 0.05 level.

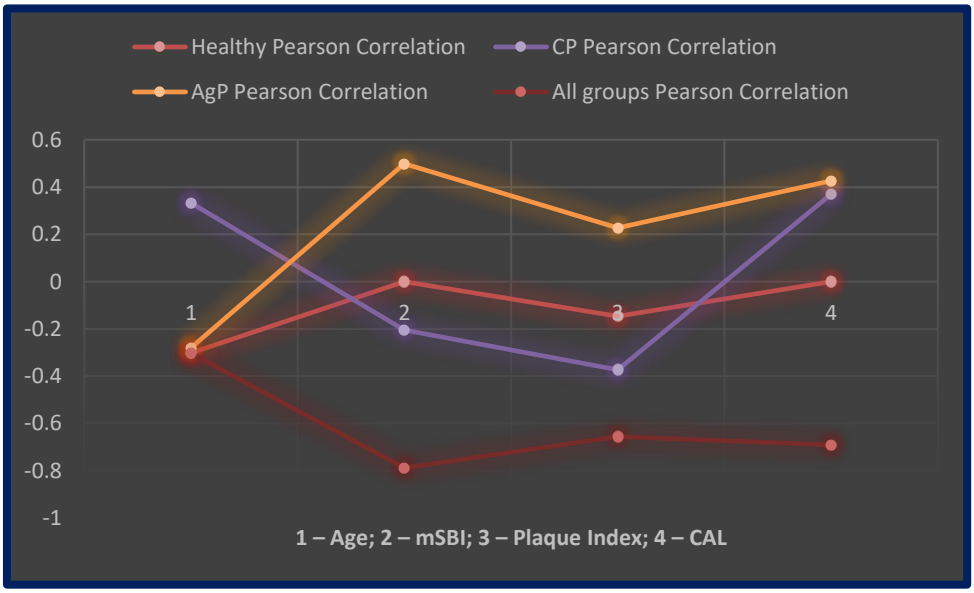
Graph 8 - Tukey HSD - Comparison of GCF POSTN values among the groups



Series1: Mean Difference; Series2: p value

*. The mean difference is significant at the 0.05 level.

Graph 9 – Pearson correlation between the levels of POSTN in GCF and age, mSBI, Plaque Index and CAL



In all groups, negative correlations were found between the levels of POSTN in GCF and age, mSBI, Plaque Index and CAL. In the healthy, negative correlations were found between the levels of POSTN in GCF and age, Plaque Index. In the CP group, negative correlations were found between the levels of POSTN in GCF and mSBI, Plaque Index. In the AgP group, negative correlation was found between the levels of POSTN in GCF and age only.

DISCUSSION

DISCUSSION

The present study consisted of three groups- healthy subjects, patients with CP and AgP. The GCF was collected from all the individuals of the study groups and was analysed by ELISA procedure for the presence and level of POSTN. The levels of POSTN in GCF was compared between and among the groups and was correlated with the clinical parameters recorded. POSTN has been shown to be an important regulator of bone formation.[7] Despite its preliminary description in bone, its biological functions are also essential for connective tissue integrity in both health and disease.[8] POSTN functions as a matricellular protein in cell activation by binding to their receptors on cell surface, thereby exerting its biological activities.[9] POSTN, secreted by fibroblasts, is found to be present in various tissues, serum, saliva and also GCF. GCF POSTN levels have been found to decrease in relation to the progression and severity of CP[10] and also AgP.[11] POSTN can act as a novel biomarker for the periodontal disease activity and healing tissues.

The sample size calculated was 12 in each group. One extra sample was collected in each group as a standby for avoiding any errors while processing and hence the total number of samples was 13 in each group.

In the present study, the mean age in healthy, CP and AgP groups was 24.69, 43.46 and 26.85 years respectively (Table 3) with a statistically significant difference between all the groups. When the mean age was compared among groups, there were statistically significant differences among healthy and CP groups, among CP and AgP groups and there was no statistically significant difference among healthy and AgP groups. Similarly, in **Aral *et al*.'s (2016)** study, the mean age was significantly higher in the CP (38.22 years) than in the AgP group (32.17 years) ($p < 0.05$). [11]

The mean Plaque Index score was 0.24, 1.18 and 0.96 in healthy, CP and AgP groups respectively in the present study (Table 4). There was a significant difference between all the groups. When the mean Plaque Index was compared among groups, there were statistically significant differences among healthy and CP groups, among healthy and AgP groups and there was no statistically significant difference among CP and AgP groups. In **Aral *et al.*'s (2016)** study, CP (1.66) and AgP (1.85) patients presented with higher mean Plaque Index scores than the non-periodontitis (0.78) group ($p < 0.001$); however, no statistically significant differences were observed between CP and AgP groups ($p > 0.05$), [11] which was in accordance with the present study. In **Balli *et al.*'s (2015)** study, there was a statistically significant difference ($p < 0.016$) in the mean Plaque Index score between the healthy (0.49) and CP (2.30) groups which is similar to the results of the present study. [10]

The mean values of mSBI percentage for the healthy, CP, AgP groups were 0%, 65.7% and 69.7% respectively (Table 4). When the mSBI was compared among groups, there were statistically significant differences among healthy and CP groups, among healthy and AgP groups and there was no statistically significant difference among CP and AgP groups. Similar to the present study, no statistically significant difference in full-mouth bleeding on probing score was observed between CP (55.35%) and AgP groups (58.99%) ($p > 0.05$) in **Aral *et al.*'s (2016)** study. [11]

The mean values of CAL for the healthy, CP, AgP groups were 0, 4.5 mm and 5.17 mm respectively (Table 4). There was a statistically significant difference between the groups. When the mean CAL was compared among groups, there was a statistically significant difference among healthy and CP groups, among healthy and

AgP groups and it was not statistically significant among CP and AgP groups. Similarly, in **Aral *et al.*'s (2016)** study, there was a statistically significant difference in the mean CAL value among healthy (0.18 mm) and CP (3.82) groups and among healthy and AgP (4.13) groups. [11]

Compared to healthy and CP groups, GCF POSTN levels in the AgP group (49.28 pg/µl) were the lowest. Moreover, GCF POSTN levels in the CP group (79.87 pg/µl) were lower than in the healthy group (182.41 pg/µl) ($p < 0.05$). Similar to the present study findings, **Aral *et al.* (2016)** investigated GCF POSTN levels in CP and AgP patients and found that there was a significant reduction in GCF POSTN levels in CP (0.58 pg/30s) and AgP (0.46 pg/30s) groups when compared to non-periodontitis (0.72 pg/30s) group. [11] **Balli *et al.* (2015)** investigated GCF POSTN levels in healthy subjects (346.93 pg/µl), gingivitis (108.86 pg/µl) and CP patients (51.64 pg/µl) and concluded that GCF POSTN levels decreased in relation to the progression and severity of periodontitis, [10] which is in accordance with the present study.

The present study results, therefore, confirmed a negative correlation between GCF POSTN levels and periodontal diseases. In this study, the reduction in GCF POSTN levels seen in CP and AgP groups may explain the delayed tissue repair observed in the inflammation of periodontal tissues. There were significant differences among all three groups in GCF POSTN levels and it was found to be highly reduced in the AgP group compared to the CP and healthy groups, suggesting a role for POSTN in the disease activity in periodontal diseases. In a study conducted by **Kumaresan *et al.* (2016)**, a statistically significant difference in the concentration of POSTN in GCF was found between healthy controls (6.54 ng/ml) and CP group (3.46 ng/ml) ($p < 0.01$). [96] Therefore, as expected, we observed that the degree of inflammation and tissue

damage affected the POSTN levels in GCF potently and inversely. The decreased level of POSTN in GCF in the diseased groups might suggest that this novel molecule takes part in maintaining normal periodontal tissue function. [10]

In a study conducted by **Padial-Molina M *et al.* (2015),** the levels of POSTN in GCF/wound fluid (in pg/μl) increased over time and correlated with the healing process in both healthy and disease patients. Post hoc tests using the Bonferroni correction revealed that POSTN levels previous to the surgery [367.85 (418.87)] were statistically significantly lower than those at 48 h [1496.14 (747.48), $p < 0.001$] and 2 weeks [836.80 (199.606), $p < 024$]. It is found in the **Padial-Molina M *et al.*'s (2015)** study that GCF POSTN levels were lower in diseased condition than in healthy, similar to the present study and increased during healing after treatment.[94] The reduction of POSTN levels directly affects repair and formation potential of the periodontal tissue. [18, 61]

In the present study, there was a negative correlation found between mSBI and GCF POSTN value when compared in all groups (r = -0.788), CP group (r = -0.204) (Table 12). These results were similar to **Aral *et al.*'s (2016)** study, in which there was a negative correlation found between full mouth bleeding on probing score and GCF POSTN value when compared in all groups (r = -0.580)**.** [11]

The Plaque Index scores were negatively correlated with the levels of POSTN in GCF when compared in all groups (r = -0.655), healthy (r = -0.146) and CP (r = -0.373) groups (Table 12), in the present study, which is in accordance with the study conducted by **Aral *et al.* (2016),** where there was a negative correlation found between Plaque Index score and GCF POSTN value when compared in all groups (r = -0.505)**.** [11]

There was a negative correlation found between CAL and GCF POSTN value when compared in all groups (r = -0.691), in the present study and it was significant at 0.01 level (Table 12). These results were similar to **Aral *et al.*'s (2016)** [11] study, in which there was a negative correlation found between CAL and GCF POSTN value when compared in all groups (r = -0.581)**.**

In the present study, a positive correlation between CAL and the levels of POSTN in GCF was found in CP (r = 0.370) and AgP (r = 0.427) groups (Table 12). But, in **Balli *et al.*'s (2015)** [10] study, a negative correlation (r = -0.712) between CAL and the levels of POSTN in GCF was found in CP group. **Baeza *et al.* (2016)** also reported that, with disease severity, that is, with increase in CAL, there was decrease in GCF POSTN levels. [95]

The amount of POSTN in GCF decreased by 56% in CP group and by 73% in AgP group when compared to healthy group. In **Balli et al's (2015)** [10] study, the amount of POSTN in GCF decreased by 85% in CP group when compared to healthy group and in **Aral et al's (2016)** [11] study, the amount of POSTN in GCF decreased by 19% in CP group and by 36% in AgP group when compared to non-periodontitis group.

Hence, by assessing the POSTN concentration in GCF of patients with diseased condition and comparing with the healthy controls, early detection of the diseased states is easy and simple. The limitations of this study include smaller sample size and also not assessing the POSTN levels after treatment, since the levels of POSTN in GCF was found to be higher in healing tissues.[94] Further studies can be performed to detect the POSTN levels in GCF or tissues in CP and AgP patients after non-surgical and surgical treatments. Further newer research on chair side POSTN detection kit will

be of great help to make the procedure easier, simpler and time saving. In addition to being an inflammatory marker for periodontal disease, the present study findings may put forward the idea that increasing POSTN levels or preventing POSTN reduction may result in faster tissue repair and more attachment gain.

SUMMARY AND CONCLUSION

SUMMARY AND CONCLUSION

In the present study, the POSTN level in GCF of CP and AgP patients were assessed and compared with that of healthy subjects. A total of 39 subjects were included in the study. The GCF samples were collected using microcapillary pipette technique and analysed for POSTN by ELISA procedure.

The mean levels of total POSTN in GCF were assessed and it was found to be the highest in the healthy, lower in the CP group and the lowest in the AgP groups. There was a statistically significant difference in GCF POSTN levels between the groups. There was a statistically significant difference in GCF POSTN levels among healthy and CP groups, among healthy and AgP groups and also among CP and AgP groups.

When the healthy, CP and AgP groups were examined together, there was a statistically significant negative correlation between POSTN levels in GCF and inflammatory changes, that is, the POSTN levels in GCF decreased with increase in mSBI, Plaque Index, CAL. The high levels of POSTN in GCF in healthy group indicates its importance in maintenance of integrity of tissues and increasing POSTN levels may help in healing of diseased tissues.

From this study, the following conclusions were drawn,

- POSTN was present in each GCF sample of all the study groups and it's level was found to be the highest in the healthy group, lower in the CP group and the lowest in the AgP group.
- The mean levels of total POSTN in GCF decreased with increased severity of the disease.
- The POSTN levels in GCF and inflammatory changes were inversely related.

When the results in the present study are considered in conjunction with those of previous reports, it might be concluded that the POSTN level in GCF can be considered as a reliable marker in the diagnosis of periodontal diseases and disease activity.

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